ANIMALS

Research across a broad range of animal systems areas is urgently needed for the future enhancement of animal production efficiency, as well as to address such areas as the modification of animal products. To accomplish this, grants are awarded in four broad areas of research: (1) animal reproductive biology, (2) cellular growth and developmental biology of animals, (3) animal molecular genetics and gene mapping, and (4) animal health and well-being. Emphasis is given to innovative approaches to research questions related to animals primarily raised for food or fiber. This includes aquaculture species and those animals such as horses that contribute significantly to the agricultural enterprise of the country.

ENHANCING ANIMAL REPRODUCTIVE EFFICIENCY

Panel Manager - Dr. Duane Keisler, University of Missouri
Acting Program Directors - Dr. Carl V. Profater III, Dr. William Wagner

The primary objective of this program area is to increase our knowledge of reproductive biology in agriculturally important animals with the goal of increasing reproductive efficiency. This program will consider for support innovative research on: (1) mechanisms affecting embryo survival, endocrinological control of embryo development, mechanisms of embryo-maternal interactions, embryo-implantation, and development of optimal embryo culture methods, (2) factors controlling ovarian function including follicular development, ovulation, and corpus luteum formation and function, (3) factors controlling male reproductive function, (4) gamete physiology, including oogenesis and spermatogenesis, gamete maturation, mechanisms regulating gamete survival in vivo or in vitro, and (5) parturition, postpartum interval to conception, and neonatal survival. Proposals are also encouraged on fundamental mechanisms involved in placental function and separation or causes and remediation of early embryonic loss.

Because alterations in animal behavior and animal well-being may impair fecundity, this program also encourages research on the mechanisms controlling animal responses to physical and biological stresses that impinge upon reproductive processes. Research should contribute to an understanding of the causes, consequences, and avoidance of stress, rather than merely describing the physiological effects of stress on reproductive efficiency.

9703178 Use of Liposomes to Cryopreserve Rooster Sperm
Graham, J.K. **Grant 97-35203-4609**

Colorado State University
Department of Physiology **$130,000**
Fort Collins, CO 80523 **2 Years**

Fertility of cryopreserved bull sperm is satisfactory, but similar procedures have not resulted in practical preservation of sperm from boars, rams, turkeys or roosters. The reason sperm from these species are infertile after cryopreservation is not known. However, solution of the problem would save the poultry industry alone, > $25 million annually. Part of these savings would result from enhanced genetic selection of males for increased growth rate and part from conserving valuable genetic poultry strains "in the freezer" rather than maintaining large numbers of birds.

Since sperm membrane damage is a major problem during cryopreservation, much of which results from osmotic stresses caused by cryoprotectant movement across the plasma membrane, we will investigate the movement of glycerol across the plasma membrane of rooster sperm pretreated with liposomes to alter membrane permeability.

The objectives of this research are: 1) determine if exposure of rooster sperm to liposomes increases sperm membrane permeability to glycerol and improves sperm survival after cryopreservation; 2) develop a procedure to reduce glycerol concentration in the inseminate without inducing osmotic damage to sperm; and 3) determine if methyl cellulose can reduce the contraceptive effects of glycerol sufficiently to preclude glycerol removal from the inseminate prior to insemination. Four analytical techniques will be used: flow cytometry, to evaluate membrane permeability to glycerol and to evaluate cell viability; computerized motion analysis to evaluate sperm motion parameters; fluorescent microscopy to evaluate sperm capacity to bind to and penetrate the perivitelline membrane in vitro; and fertility trials to evaluate the fertilizing potential of cryopreserved rooster sperm.

9702319 Mechanisms Regulating the Mid-luteal Phase Increase in Secretion of FSH in Cows
Nett, T. **Grant 97-35203-4855**

Colorado State University
ARBL **$182,779**
Fort Collins, CO 80523-1683 **3 Years**

There is a great deal of variation in the quality of beef available to the American consumer, much more so than the quality of poultry or pork. One reason for the consistency of product in the poultry and pork industries is the widespread use of artificial insemination which leads to reduced genetic variability. To date artificial insemination (AI) has not been widely adopted by beef producers. The reason for this is that most beef cattle are maintained under range conditions rather than under intensive management. It is not economically feasible to use AI under range conditions unless cows can be inseminated at approximately the same time. Therefore, for AI to be economically feasible for most beef producers, it must occur after estrous synchronization. Unfortunately, there is a great deal of variation in the time that cows may show estrous behavior (12-120 hour) after a treatment to synchronize estrus. This degree of variation in the onset of estrus increases the cost of AI to the point that most beef producers find it uneconomical. The primary reason for this variation is that ovarian follicles require several days for final maturation and we have not been able to synchronize follicular maturation. Thus, it is imperative to gain a better understanding of the factors controlling follicular growth and maturation. Such information may lead to new treatments that reduce the variability associated

with the onset of estrus after a synchronization treatment, thus making AI more feasible for the beef producer. In the long run, this will lead to a much more consistent product for the American consumer.

9703049 Endothelin-1 and Bovine Luteolysis
Milvae, R.A. **Grant 97-35203-4856**

University of Connecticut
Department of Animal Science, U-40
Storrs, CT 06269-4040

Strengthening Award
$140,000
2 Years

The long term goal of this research is to gain a better understanding of the processes controlling regression of the bovine corpus luteum (CL). Previous studies and results of our preliminary experiments led to the immediate objective of this proposal which is to test the hypothesis that endothelial cells and their biosynthetic product, endothelin-1 (ET-1), play an important luteolytic role in bovine CL function, at least in part, by mediating the effects of PGF. In the proposed experiments, we will test this hypothesis utilizing luteal cells obtained from heifers at different stages of the estrous cycle and via intraovarian administration of ET-1 and ET-1 antagonists. The specific aims are to 1) determine numbers of luteal endothelial cells, luteal content and synthesis of ET-1, and expression of ET-1 and its receptors (ETA) in luteal tissue during the estrous cycle and early pregnancy, 2) clarify the cellular mechanisms by which ET-1 inhibits progesterone production, 3) determine if ET-1 injected directly into the CL induces luteolysis and whether ET antagonist administration prevents spontaneous and PGF-induced luteal regression and 4) determine luteal secretion of ET-1 during spontaneous and PGF-induced luteolysis. The net results of these experiments should yield valuable new information on the cellular mechanisms involved in luteal regression. These results will lead to development of better methods of fertility control in the cow, improved techniques for estrous cycle synchronization and decreased losses due to embryonic mortality in all domestic species.

9702910 Function of Oviductal Secretory Protein(s)
Buhi, W.C.; Wielbo, D. **Grant 97-35203-4614**

University of Florida
Department of Obstetrics and Gynecology
Gainesville, FL 32610-0294

$220,000
3 Years

The long-term goals of this research are to increase our knowledge of oviductal function and understand how specific oviductal constituents are involved in fertilization and early cleavage-stage embryonic development in the pig. Studies clearly show that the porcine nonciliated oviductal epithelium synthesizes and releases three major proteins. One, porcine oviductal secretory glycoprotein (POSP), is estrogen-dependent. Two other proteins, tissue inhibitor of metalloproteinase (TIMP)-1 and plasminogen activator inhibitor (PAI)-1, are major proteinase inhibitors also synthesized and released by the oviduct. POSP has been purified, sequenced, and cloned and shown to be similar to proteins found in the oviducts of other species. Localization studies at the molecular level demonstrate that POSP associates with the ovulated egg and early embryo before implantation. This conservation of structure across species and association with gamete and embryo suggests an important function for POSP in early pregnancy. Therefore, in this study, we will examine the role of POSP on sperm penetration and fertilization of the oocyte and its potential role in early embryonic development and metabolism in an in vitro fertilization system. To complement this study, we will develop an in vitro/in vivo system to inhibit the specific synthesis of POSP and ultimately show in vivo that POSP plays a significant role in fertilization and/or early embryonic development.

Failure of reproduction and development is a significant problem in animal reproduction and the oviduct occupies a central role in influencing reproductive events. At present, our knowledge of oviductal biology and function are inadequate. The proposed research will significantly add to our understanding of the role of the oviduct and oviduct-specific proteins in oviductal egg and oviductal embryo interaction. This may allow development of methods to improve or manipulate fertilization and embryonic development.

9702256 Role of Estrogen in Epididymal Function of the Rooster
Bahr, J.; Bunick, D.; Hess, R.A.; Helman, S. **Grant 97-35203-4615**

University of Illinois
Department of Animal Science
Urbana, IL 16801

$195,000
3 Years

Proper functioning of the male reproductive tract is essential for fertility. Sperm, produced by the testis, become fertile as they pass through the reproductive tract. Therefore, an understanding of the regulation of the male reproductive tract is essential.

Traditionally, it has been assumed that androgen, the male steroid hormone, regulates the male reproductive tract. We are proposing that estrogen, the female steroid hormone, is also required. Our hypothesis is based on several discoveries we have made recently. First, we found that sperm can make estrogen. Second, we found that there are a large number of receptors for estrogen in the male reproductive tract, suggesting that the tract is a target for estrogen.

Our current goal is to test the hypothesis that estrogen regulates fluid resorption by the reproductive tract. Approximately 90% of the fluid which enters the reproductive tract from the testis is resorbed. This resorption is necessary to provide the optimal fluid environment for maturation of sperm. Fluid resorption is a passive process, controlled by the movement of small molecules, such as sodium, chloride. In our proposed studies we will determine if this regulation of the movement of these small molecules and indirectly fluid is controlled by estrogen.

This research is important because of the importance of proper functioning of the male reproductive tract for fertility. The control of essential functions by estrogen is specifically important because of the concern of estrogen like compounds in our environment which may alter the function of the reproductive tract and thus alter fertility of the male.

9702343 Characterization of a Family of Ovarian and Ovulation Specific Proteins in Trout
Goetz, F.W. **Grant 97-35203-1962**

University of Notre Dame
Department of Biological Sciences
Notre Dame, IN 46556
$190,000
3 Years

Aquaculture is one of the fastest growing industries in North America. In the future, to meet the demands of this industry a greater emphasis will have to be placed on controlling fish reproduction. A major problem for the artificial reproduction of fish in aquaculture is the maintenance of the quality of eggs obtained from females. If eggs are not fertilized quickly, they lose viability. Thus, techniques or products that can be used to maintain egg viability would benefit the aquaculture industry. We are investigating a protein that is produced in the brook trout ovary and is secreted in high concentration into the ovarian fluid that surrounds the eggs once they are released from the ovary. We have characterized this protein in several ways and believe that it is an *inhibitor* of degradative proteases involved in the release of eggs at that time of spawning. Left unchecked, these proteases would damage the egg and decrease viability. In trout, the released eggs are held within the body cavity in the ovarian fluid for several days without loss of viability. Since the protein we are studying is present in such high concentrations in the ovarian fluid, a natural function of this protein may be to maintain egg viability prior to spawning. Thus, this protein might also be very useful in maintaining egg quality during the artificial reproduction of aquacultured fish.

9702919 Signaling Pathways in the Maturation of Bovine Oocytes: Role of Map Kinase and Calcium
Fissore, R. **Grant 97-35203-4907**

University of Massachusetts
Paige Laboratory
Amherst, MA 01003
$110,000
3 Years

This proposal will investigate the role of MAP Kinase (MAPK) and calcium ($[Ca^{2+}]i$) in bovine oocyte maturation, MAPK, which is part of a kinase cascade, has been shown to be involved in spindle organization, polar body extrusion, and meiotic arrest. MAPK function in oocytes will be blocked by injection of mRNAs encoding either for a kinase inactive MEK, a direct activator of MAPK, or for a CL-100 phosphates, that directly inhibits MAPK activation. Their effects on meiosis resumption and metaphase II arrest will be determined by monitoring the status of chromatin of injected oocytes. Calcium may participate in the activation of kinases during maturation. SERCA2b mRNA, a Ca^{2+}ATPase pump, will be overexpressed in oocytes and its effects on $[CA^{2+}]i$ oscillations during maturation and fertilization, activation of kinases, and progression through meiosis will be assessed. Lastly, the spatial location of two isoforms of the 1,4,5-inositol-triphosphate receptor ($InsP_3R$) will be investigated. $InsP_3Rs$ are present in oocytes and their spatial redistribution during maturation may contribute to their increased ability to release Ca^{2+} as oocytes reach metaphase II. Thus, we propose to investigate the role of MAPK and Ca^{2+} on the initiation, progression, and arrest of meiosis in bovine oocytes. Understanding their functions, targets, and spatial distribution will allow us the establishment of biochemical and molecular markers to monitor cytoplasmic maturation and, possibly, the acquisition of meiotic and developmental competence. This should contribute to elucidate cellular deficiencies in oocytes with poor developmental capacity and may enhance the outcome of IVM and IVF procedures.

9702266 Regulation of Bovine Sperm Aster Formation
Robl, J.M. **Grant 97-35203-4905**

University of Massachusetts
Department of Vet. & Ani Science
Amherst, MA 01003
$120,000
4 Years

Following penetration of the egg at fertilization the sperm head and tail separate. The anterior tip of the sperm tail contains a centriole, which becomes organized as a centrosome, and initiates polymerization of an aster of microtubules. These microtubules act as structural elements and guide the male and female pronuclei to a position, tightly opposed to one another, in the center of the egg. The goal of this study is to investigate the regulation of sperm centrosomal function in bull sperm. The hypothesis to be tested is that structural proteins surrounding the sperm centrosome must be disassembled by dephosphorylation

and, possibly disulfide reduction, before the centriole can form an active centrosome. In addition, the proposal will investigate the importance of the centrosome following sperm injection into the egg; particularly in cases where the sperm is not fully formed. Fertilization by sperm injection would allow the use of small populations of sperm, such as sorted sperm, for sex selection or the use of immature testicular sperm to reduce the generation interval in cattle.

9702298 Gamete Biology: Fundamental and Applied Aspects in Animal Reproduction
Overstrom, E.W.; Albertini, D.F. **Grant 97-35203-4961**

Tufts University
School of Veterinary Medicine **$5,000**
North Grafton, MA 01536 **1 Year**

This project will support the travel and living accomodations for a group of invited speakers at a mini symposium to be held in conjunction with the International Embryo Transfer Meeting to be held in Boston, MA in January of 1998. The subject of the symposium is Gamete Biology: Fundamental and Applied Aspects in Animal Reproduction. Remarkable advances have been made in recent years on the conditions that are required in vivo to sustain the development and maturation of mammalian gametes. Only recently has it been possible to manipulate and control aspects of gamete differentiation using in vitro methodologies that would improve means for assessing gamete quality as well as provide alternatives to cryopreservation. The group of speakers proposed to participate in this mini symposium have made important contributions in the development of culture and cryopreservation techniques in experimental and domestic species. Their contributions to this meeting should enhance the research programs of attendees with respect to the more widespread application of these emerging methods in large animal reproduction.

9702210 Thyroid Hormones and Seasonal Reproduction
Karsch, F.J. **Grant 97-35203-4908**

University of Michigan
Department of Developmental/Reproductive Biology **$220,000**
Ann Arbor, MI 48103 **3 Years**

This research deals with fertility regulation in a farm animal, the female sheep. The broad objective is to understand the mechanisms that underlie termination of the breeding season, which recurs each year throughout the life of the animal. This annually occurring end of reproductive activity constrains breeding programs and reproductive efficiency in a number of farm animals. The end of the breeding season results from changes in neuroendocrine function. The most important of these is a decrease in the secretion of a hypothalamic hormone, gonadotropin releasing hormone (GnRH). At another level, hormones secreted by the thyroid gland are obligatory for the seasonal decrease in GnRH. In the absence of thyroid hormones, the non-breeding season is eliminated and sheep remain fertile year round. The action of thyroid hormone on seasonality is exerted only during a critical period of sensitivity and preliminary evidence suggests the action of thyroid hormone is exerted directly within the brain. The specific objectives of this project are to complete a test of the hypothesis that thyroid hormones act directly within the brain, and then to investigate neural mechanisms that mediate thyroid hormones action. For this latter point, experiments will test the hypothesis that thyroid hormones impact upon the activity of a neural system known to inhibit GnRH secretion on a seasonal basis. This system produces the neurotransmitter dopamine in cells located a restricted region of the hypothalamus and releases this neurotransmitter around terminals of neurons that release GnRH. The studies have considerable impact for enhancing reproductive efficiency in seasonally breeding farm animals. Results should lead to new approaches for altering timing of the fertile season and perhaps to overcome the infertile period altogether.

9702443 Pituitary Control of FSH Secretion
Padmanabhan, V.; Karsch, F.J.; Thompson, R.C. **Grant 97-35203-4906**

University of Michigan
Department of Pediatric and Reproductive Sciences Program **$220,000**
Ann Arbor, MI 48109-0718 **2 Years**

To optimize reproductive performance, it is essential we acquire a thorough understanding of the mechanisms that are involved in regulating estrous cyclicity and ovulation. In spite of the pivotal role follicle-stimulating hormone (FSH) plays in controlling follicular development, and the current utility of this hormone in breeding practices, the regulatory mechanisms involved in the control of this reproductively-important hormone is incomplete. Utilizing unique approaches, including pituitary portal sampling, microperifusion systems and sheep models, we have begun to unravel these complex regulatory mechanisms. The long term goal of this project is to elucidate the mechanisms governing the release of this crucial hormone. Our recent studies in sheep provide compelling evidence that: 1) FSH secretion is comprised of two modes of secretions a pulsatile and a basal mode; 2) the basal component is the dominant mode of FSH secretion and is not under the direct control of gonadotropin-releasing hormone (GnRH), a hypothalamic hormone released in a pulsatile manner and involved in the release of both luteinizing hormone (LH) and FSH; 3) the level of basal FSH secretion appear to be altered by changes in other FSH-regulatory proteins such as

activin, inhibin, and follistatin that are produced by the ovary; and 4) the pituitary gland which secretes FSH also produces these FSH regulatory proteins. These findings lead to a series of compelling questions: How is the basal component of FSH release regulated? Do activin, inhibin and follistatin produced in the pituitary direct the level of basal FSH secretion? Is there a role for these regulatory proteins circulating in the blood? This proposal will address these issues. The proposed studies will not only lead to clarification of the mechanisms governing FSH release, advancing our understanding of reproductive biology, but will provide vital information which can be utilized practically in the future to optimize the timing and number of ovulations as well as to suppress ovulation and regulate fertility in farm animals.

9702233 Modulation of Reproductive Efficiency by Prolactin in the Domestic Turkey
El Halawani, M.E. **Grant 97-35203-4960**

University of Minnesota
Department of Animal Science
St. Paul, MN 55108

$230,000
3 Years

Studies outlined in this proposal are aimed at elucidating the physiological mechanism(s) that initiate and maintain hyperprolactinemia in incubating female turkeys. Convincing evidence is presented implicating elevated prolactin levels as a cause of follicular stresia, termination of ovulation and cessation of egg laying. Vasoactive intestinal peptide is the only identified physiological turkey prolactin releasing factor.

Three objectives are outlined for study in the present proposal. Objective I outlines studies to identify and characterize the mechanism(s) by which vasoactive intestinal peptide regulates prolactin secretion. Pituitary prolactin transcription rates (nuclear run-on assay) ant prolactin mRNA stability (half-life) will be determined in response to vasoactive intestinal peptide *in vitro*. The effect of vasoactive intestinal peptide immunoneutralization on prolactin transcription and mRNA stability will also be examined. Experiments are designed to test the hypothesis that known prolactin secretogogues are involved in activating the central neurons and regulating the differential release of vasoactive intestinal peptide of hyper- and hypoprolactinemic turkeys.

Objective II describes experiments to characterize the neurochemical circuitry of the mechanisms regulating vasoactive intestinal peptide, and by extension, prolactin secretion. Pharmacological approaches will be utilized to investigate the involvement of dopaminergic and serotonergic neurotransmission in the control of vasoactive intestinal peptide/prolactin secretion. Specific receptor agonists and antagonists will be infused intracerebroventricularly or perfused *in vitro* (hypothalamic explants) and their effects on vasoactive intestinal peptide secretion will be determined.

Objective III will be to prevent the onset of incubation behavior and extend the egg laying season by using recombinant vasoactive intestinal peptide-fusion protein to immunize against endogenous vasoactive intestinal peptide.

Results of this research will provide novel insight into the hypothalamic and pituitary mechanisms regulating hyperprolactinemia and the induction of incubation behavior. Such information is critical for the advancement of our knowledge about avian reproductive neuroendocrinology and for the pursuit of improved reproductive efficiency in the domestic turkey.

9702442 Interferon-tau Expression and Action during Pregnancy
Ealy, A.D. **Grant 97-35203-4767**

University of Missouri, Columbia
Department of Animal Sciences
Columbia, MO 65211-5300

Postdoctoral Fellowship
$90,000
2 Years

The main goal of this work is to provide a better understanding of the actions of interferon-tau proteins. These proteins are secreted from the developing embryo of ruminant species, such as sheep and cattle, during early pregnancy and are responsible for signaling the presence of the embryo to the mother during early pregnancy, this event is required in order for pregnancy to be maintained in these species. Numerous genes encode for interferon-tau in sheep and cattle, but it remains unknown which of these genes encode for protein products that are primarily responsible for acting as pregnancy recognition signals. Through this work, the predominant variants of interferon-tau that are produced by the ovine embryo will be identified. In addition, recombinant proteins of these expressed gene variants will be produced in bacteria and will be used to identify those protein variants that are most potent at mimicking the events of early pregnancy when injected into the uterus of nonpregnant ewes. The final goal of this work is to begin to elucidate the means by which interferon-tau acts on the uterus to elicit pregnancy recognition signals, and, in particular, to identify signaling systems within the uterus that are responsive to interferon-tau. This knowledge will be very useful for increasing our basic understanding of early pregnancy in ruminants and, may lead to the development of schemes that can improve pregnancy rates in sheep and cattle.

9703061 Recombinant Bovine Gonadotropins
Grotjan, E. **Grant 97-35203-4809**

University of Nebraska
Department of Animal Science **$150,000**
Lincoln, NE 68583-0908 **2 Years**

Our ability to increase the influence of genetically superior cows is currently limited by suboptimal procedures for stimulating the development of multiples follicles for embryo transfer (routinely called superovulation). The goal of this project is to produce hormones named follicle-stimulating hormone (FSH) and luteinizing hormone (LH) with recombinant DNA technology for use in stimulating the development of ovarian follicles and luteal tissue involved in the maintenance of pregnancy in cows. The gonadotropins FSH and LH are composed of two protein chains which associate with each other to form active complexes. Recombinant bovine FSH and LH will first be made by simultaneously producing the two protein chains in either insect or mammalian cells. The actions of the hormones will be assessed by their ability to induce normal biological responses. One protein chain of the chorionic gonadotropins (LH-like hormones) produced by the placenta of primates and horse-like species has a unique chain of amino acids which prolongs its biological effects. Bovine FSH- and LH-like hormones will be produced by extending one of the two protein chains to include a region analogous to that which occurs on the chorionic gonadotropins to prolong their biological actions. Finally, rather than producing bovine FSH and LH as two protein chains, each hormone will be produced as one long protein chain. This strategy not only facilitates production using recombinant DNA technology but also yields hormones with enhanced biological activity because the hormones are more stable. The availability of recombinant bovine gonadotropins will allow development of improved methods for superovulation of genetically superior cattle.

9702277 Function and Regulation of Inhibin and its Subunits in the Domestic Hen
Johnson, P.A. **Grant 97-35203-4979**

Cornell University
Animal Science Department **$145,000**
Ithaca, NY 14853-4801 **3 Years**

Our overall goal is to understand the process of follicle selection and development in the hen. A fundamental understanding of follicular recruitment is essential to maximizing reproductive efficiency, especially in turkeys and broiler breeder hens where egg production is not optimal. From a basic biological perspective the features of the avian follicle make the hen an ideal model for understanding the process of regulation of follicular recruitment. Inhibin is a gonadal hormone involved in the regulation of pituitary FSH. By understanding the role of inhibin in a species in which preovulatory development is so ordered and predictable, it may be possible to generalize these findings to other domestic animal species as well. The follicle of the hen is large enough so that production of inhibin can be estimated from an individual follicle. In addition, the time of expected ovulation can be readily predicted from the oviposition pattern and digital palpation. We intend to investigate the local (within the ovary) effects that inhibin and related hormones have. In addition, we will examine the influence of follistatin which is a binding protein for inhibin. These hormones and their function will be examined in small and large follicles. When we understand the nature of the negative feedback between the ovarian hormone inhibin and the pituitary hormone FSH, we will know much more about follicle development in birds. This knowledge may suggest strategies to increase egg production in turkeys and broiler breeder hens.

9702913 Mechanisms Governing Movement of Sperm in the Oviduct
Suarez, S.S. **Grant 97-35203-4734**

Cornell University
Department of Anatomy **$280,000**
Ithaca, NY 14853 **3 Years**

The long-term focus of this research is to determine how sperm movement is regulated in the oviduct (fallopian tube) to ensure that fertilization occurs. When sperm reach the oviduct, they are trapped just inside the entrance, forming a reservoir. As the time of ovulation nears, a few sperm are released to meet the oocyte. There is evidence that the reservoir serves to prevent polyspermic fertilization and to maintain sperm fertility while they are in the reservoir. We have determined that an important mechanism for trapping sperm is binding them to the oviductal epithelium. This binding is via recognition by sperm of certain sugars expressed on the surface of the epithelium, much like lectin/ligand interactions. Bull sperm binding to oviductal epithelium is inhibited *in vitro* by fucose. Pretreatment of epithelium with fucosidase to remove fucose prevents binding. Therefore, fucose is involved in binding sperm to epithelium, probably as a component of glycoproteins or glycolipids expressed on the surface of the epithelium. The component on sperm which recognizes fucose on the oviductal epithelium will be referred to as a lectin. The overall objective of this particular project is to characterize the fucose lectin on bull sperm. The specific aims are: (1) *To determine the specificity of the bull sperm lectin.* Preliminary experiments indicate that the lectin only binds to fucose in certain chemical linkages to other sugars; therefore, we will test the efficacies of various fucose-containing oligosaccharides for blocking sperm binding to oviductal epithelium; (2) *To localize the lectin on the sperm.* The oligosaccharide found to be most effective

at blocking sperm binding will be conjugated to a fluorescent tag and used to label sperm. The label should be distributed over the outer surface of the plasma membrane overlying the sperm acrosome, because this is the site of attachment of the sperm to the epithelium; (3) *To determine whether the lectin is lost or modified during sperm capacitation*. Capacitation is the process by which sperm become prepared to fertilize. It involves changes in the sperm surface. Capacitation reduces binding to epithelium, therefore, detection of the lectin on sperm should diminish with capacitation; (4) *To purify and characterize the bull sperm lectin*. The oligosaccharide will be agarose beads for use in affinity purification of the sperm lectin. Activity will be assayed by binding inhibition studies and purity assayed by gel electrophoresis. Sequence data will be obtained and used to characterize the lectin. In conclusion, these studies could provide the basis for developing new fertility tests for sperm and the development of additives to semen extenders to improve sperm transport efficiency in the female.

9702249 Neuroanatomical Basis of Pulsatile GnRH Release in the Ewe
Lehman, M.N.; Goodman, R.L. **Grant 97-35203-4766**

University of Cincinnati
Department of Cell Biology, Neurobiology & Anatomy
Cincinnati, OH 45267-0521
$220,000
2 Years

Key to understanding how farm animals reproduce, is to understand how the brain controls the secretion of hormones that regulate fertility. One such essential hormone is gonadotropin-releasing hormone (GnRH). GnRH is the master hormone controlling reproduction. It is made by brain cells, and carried by specialized blood vessels from the brain to the pituitary gland, where it, in turn, controls the secretion of other hormones. GnRH plays a critical role in regulation of fertility in farm animals including sheep. Changes in the activity of GnRH brain cells control the estrous cycle, and the shut down of reproduction that occurs during seasonal anestrus and prior to puberty. Our long-term goal is to understand the signals and neurochemicals in the brain that control GnRH cells. Previous work has shown that endogenous opioids, which act in the brain to alleviate pain and stress, are also important regulators of GnRH activity. In addition, we have preliminary evidence that in the sheep brain a subset of GnRH cells is activated in response to a drug that blocks opioid receptors. In the proposed experiments, we will determine the particular type of opioid receptor that is responsible for controlling these GnRH cells, and see if that receptor is present in GnRH cells. This research will help reveal the means by which the brain normally stimulates GnRH secretion. Because changes in GnRH secretion are critical in controlling sheep reproduction, this knowledge will allow us to develop new ways of maximizing reproductive efficiency in sheep and alleviating inefficiencies in lamb production.

9702358 Microtubule Rearrangements & Centrosome Inheritance during Bovine Fertilization
Schatten, G.P. **Grant 97-35203-4613**

Oregon Health Sciences University
Oregon Regional Primate Research Center
Beaverton, OR 97006
$200,000
2 Years

Efficient reproduction depends on successful fertilization, which is completed when the parental genomes (i.e., DNA) unite within an activated oocyte. Past investigations on fertilization in domestic species has lead to the discovery that the sperm introduces a key component which is critical to the fertilization process- the centrosome, the cell's microtubules. Microtubules have many functions within the cell, including aligning and segregating chromosomes during meiosis or mitosis and positioning male and female pronuclei within the cytoplasm. This proposal will examine the proteins which partially comprise the centrosome and investigate which of these molecules are essential for completion of fertilization. A second goal is to explore the steps involved in the migration of the male and female pronuclei using concentrated frog cytoplasmic extracts which permit imaging and manipulation of microtubule assembly from the introduced sperm centrosome. Together, the information obtained during these experiments will increase our understanding of a crucial, but poorly understood, step in the reproductive process of all animals- the mechanism leading to pronuclear apposition and genomic union during fertilization. The knowledge garnered may provide new discoveries for testing male reproductive capability, assaying compounds which might affect fertility and improving reproductive efficiency in a commercially important domestic species.

9702500 Heritability & Basis of a New Reproductive Trait in Male Poultry
Froman, D.P. **Grant 97-35203-4807**

Oregon State University
Department of Animal Science
Corvallis, OR 97331-6702
$185,000
3 Years

The U.S. broiler industry is a multi-billion dollar agribusiness. Broiler production depends upon intense genetic selection and amplification of bird numbers; thousands of highly selected breeders are used to generate billions of broilers over the course of 5 generations. Traditionally, breeder males have been chosen only on the basis of growth and body shape. The principal investigator (PI) has discovered a laboratory test that measures the size of a highly mobile subpopulation of sperm within a

rooster's ejaculate. The PI has discovered extreme variability in sperm mobility among normal, fertile males. Furthermore, the PI has demonstrated that sperm mobility is a trait that: (1) can be used to categorize individual roosters, (2) is predictive of the number of chicks that can be obtained from a rooster, (3) might be highly heritable, and (4) depends upon the extent to which sperm cells generate the kind of chemical energy needed for cellular motility. The proposed research will determine the extent to which the trait is heritable and the cellular property that accounts for the trait. To date, sperm mobility has been an essential but uncontrolled variable in poultry production. However, sperm mobility may be subject to control through genetic selection in addition to choosing certain males as breeders. In either case, anticipated outcomes are: (1) changes in breeder management that will increase the reproductive efficiency, and (2) the emergence of poultry semen as a commodity due to the formulation of objective, biologically-significant criteria that can be used to evaluate semen quality.

9702426 PGF_{2a}-Induced Regulation of MARCKS Protein in the Bovine Corpus Luteum
Stormshak, F. **Grant 97-35203-4681**

Oregon State University
Department of Animal Sciences **$210,000**
Corvallis, OR 97331-6702 **3 Years**

In the nonpregnant cow the demise of the corpus luteum, which initiates the onset of another estrous cycle, is the consequence of a double positive feedback system between the corpus luteum oxytocin and uterine prostaglandin F (PGF). Oxytocin released in response to systemic PGF promotes additional secretion uterine PGF, which ultimately causes the death of the corpus luteum. Commercial preparations of PGF used to synchronize estrus are only effective after the corpus luteum is well developed. Why PGF administered to nonpregnant cows is not 100% effective in causing regression of the corpus luteum is not understood. The objective of the proposed research is to examine the biochemical changes of a protein inside the luteal cell which plays an important role in facilitating the actual release of oxytocin from the cell. This protein, whose function is indirectly regulated by PGF via the enzyme protein kinase C, is referred to as the MARCKS protein. Research will be conducted to evaluate the changes in corpus luteum concentrations of the MARCKS protein during various stages of the estrous cycle and examine the relationship of phosphorylated MARCKS to the actual secretion of oxytocin. Research will also be conducted to identify the species of protein kinase C that phosphorylates MARCKS and whether activators of PKC such as phorbol ester and calcium ionophore can provoke phosphorylation of the protein. Results of this research may lead to improvements in using commercial PGF to synchronize estrus in cattle and provide insight relative to mechanisms involved in secretion of oxytocin and other peptide hormones.

9702339 Male Factors Affecting Fertility of Bovine Sperm
Killian, G. **Grant 97-35203-4806**

The Pennsylvania State University
Department of Dairy & Animal Science **$250,000**
University Park, PA 16802 **3 Years**

Inefficient reproductive performance of dairy and beef cattle accounts for major economic losses to the producer and significantly increased costs to the consumer. Although adequate information exists to document the magnitude of the problem concerning poor reproductive performance in cattle, fundamental questions remain as to the causes of infertility. The large data base available on the fertility of dairy bulls used in artificial insemination (AI) is unique among all mammalian species. These data clearly indicate that "fertile" dairy sires with "normal" semen characteristics vary in fertilities by as much as 25%. The consequence of inseminating cows with semen from lower fertility bulls is reduced herd fertility, more "days open" and decreased profitability. Previous studies we have conducted with bulls and studies for a variety of other species suggest that seminal plasma, or its components, may affect sperm fertility. However, experiments demonstrating that a specific protein in seminal plasma directly affects the fertility of sperm have not been done.

During the past three years we identified and established purification methods for osteopontin (SP55) and lipocalin type prostaglandin D synthase (SP26), two proteins in seminal plasma that are associated with higher fertility bulls. We now propose to demonstrate experimentally whether these proteins, in native and recombinant forms, are capable of affecting specific endpoints of sperm function and male fertility. These studies will be conducted with cauda epididymal sperm recovered from cannulae indwelling in the vase deferential of bulls of known fertility. We will assess affects of SP55 and SP26 on sperm motility, viability, capacitation and the acrosome reaction, sperm-egg binding, penetration of zone-free oocytes and in vitro embryo development. Finally, we will determine whether functional parameters and fertility of ejaculated sperm can be improved by addition of SP55 and SP26 to test the feasibility of enhancing sperm fertility during semen processing. Collectively, these studies will demonstrate how parameters of sperm function and fertility are altered by SP55 and SP26. They will set the stage for understanding mechanisms of protein action in sperm fertility and explore the potential of enhancing bovine male fertility commercially.

9702257 Gordon Research Conference on Fertilization and Activation of Development
Nuccitteli, R.L.; Myles, D.G. **Grant 97-35203-4142**

University of Rhode Island
Department of Molecular and Cellular Biology
West Kingston, RI 02892-0984
$4,500
1 Year

The conference will be held July 27-August 1, 1997, at the Holderness School, Plymouth, New Hampshire. There will be nine sessions in the conference, following the Gordon Research Conference format: 1) The mechanism of sperm activation; 2) Penetration of egg surface coats and sperm-egg binding; 3) Initiation of egg activation; 4) Egg activation pathways; 5) Plenary Lecture by Ryuzo Yanagimachi; 6) Membrane fusion mechanisms; 7) The cell cycle and early development; 8) Current topics and contributed papers; 9) Integrins and disintegrins in fertilization. This will be an international meeting with speakers and discussion leaders from Europe (9), the Mid East (1), Far East (3) and Central America (1), as well as the U.S. (24). Among these 37 speakers and discussion leaders are six women and three ethnic minorities.

9702279 Biogenesis and Function of the Outer Acrosomal Membrane of Bovine Spermatozoa
Olson, G.E. **Grant 97-35203-4610**

Vanderbilt University
Department of Cell Biology
Nashville, TN 37232
$210,000
3 Years

Mammalian fertilization requires complementary recognition molecules on sperm and the extracellular coating (zona pellucida) of the egg which promote their specific binding. Once binding is established, the spermatozoon is stimulated to secrete a series of enzymes that digest the zona pellucida and permit its access to the egg surface where the gametes fuse to achieve fertilization. Recent research has made significant contributions to our understanding of the mechanisms of gamete binding and fusion, however the mechanisms driving the secretory process, which is termed the acrosome reaction, of the spermatozoon are poorly understood. The acrosome reaction requires the entry of calcium ions into the sperm which promotes the fusion of two distinct sperm membranes permitting release of the intracellular enzyme stores for zona penetration. Thus the timing, the location and the regulation of the acrosome reaction are critical to mammalian fertilization.

The objectives of this project are to define the role of calcium-binding proteins and specific regulatory molecules in the membrane fusion process of the bovine sperm acrosome reaction. We have identified a calcium-binding protein localized to the acrosomal membrane and the proposed experiments will establish its amino acid sequence, its protein-protein interactions and its role in calcium-regulated membrane fusion. Secondly, we have specifically localized the regulatory subunit of Type I cAMP-dependent protein kinase to the acrosomal segment of bovine spermatozoa and the proposed experiments will identify the targeting mechanisms which establish its localization and address its regulatory function in the acrosome reaction.

These studies will provide new insights into the molecular pathways regulating the membrane fusion events of the bovine sperm acrosome reaction. These data could lead to strategies applicable to artificial insemination and IVF both for preventing premature acrosome loss and for regulating the timing of the acrosome reaction to achieve maximal fertilization rates.

9703065 Estrogen Receptor and Gonadal Sex Differentiation: Tests of A Model for Catfish
Patino, R. **Grant 97-35203-4805**

Texas Tech University
Department of Biological Sciences
Lubbock, TX 79409-3131
$170,000
3 Years

Freshwater aquaculture has grown close to a one-billion-dollar industry. Channel catfish comprise about one half of this industry making it the most valuable food fish in the United States. It is well-established that male catfish grow faster than females, being 12-15% heavier by the time the fish reach the average market size. Thus, although the ability to culture all-male stocks of catfish would be of obvious economic value to the fish culturist, at present there are no methods for the sex identification of juvenile catfish so that manual segregation of the sexes for monosex culture is not feasible. Moreover, methods for the direct masculinization of genetic female catfish by hormonal or any other treatments are unavailable.

The mechanisms of gonadal sex differentiation in non-mammalian species, including catfish, are poorly understood. The purpose of this study is to clarify the hormonal regulation of gonadal sex differentiation in channel catfish. The focus is on feminizing steroids and their receptors. We will tests hypotheses about the role of steroids and their receptors that were derived from earlier research in our laboratory. We anticipate that an understanding of how the female sex is formed will allow the establishment of techniques for sex-reversing genetic females into males. The basic mechanisms of gonadal sex differentiation may be shared among fishes and perhaps other animals except mammals. Thus, our findings may be of general value to understand the process of sex differentiation and devise techniques of sex control in non-mammalian species with sex-linked, desirable agricultural traits. The results of our study have the potential of providing significant contributions to the agricultural production of catfish and other animals.

9702446 Calcium Storage in the Head of Bull Sperm: Physiological Importance
Parrish, J.J. **Grant 97-35203-4612**

University of Wisconsin, Madison
Department of Animal Sciences
Madison, WI 53706

$130,00
2 Years

In animal agriculture it is essential that males be of high fertility as decreased fertility results in decreased production by females and can be one of the most limiting factors producers face. Most of the research on decreased fertility has been focused on the female with little attention to the male despite the large impact that individual males can have. This proposal focuses on understanding the molecular events that occur during capacitation to modify a sperm to undergo capacitation. Specifically, it is proposed that sperm take up calcium during capacitation and load an intracellular calcium store within an organelle of the sperm head. The proposal will examine which organelle is loaded with calcium, the mechanisms used to load and release calcium from the store, and how cytoplasmic calcium levels are maintained. The effect of capacitation and cryopreservation on these mechanisms will then be investigated. This research is particularly important to the US dairy cattle industry as a majority of the females are bred artificially with cryopreserved sperm. Even minor variations among bulls in fertility of their cryopreserved semen can cause millions of dollars in lost production due to failure of females to conceive. This research will lay the foundation for examining if alterations to basic molecular mechanisms of capacitation lead to differences in the fertility of cryopreserved semen. In the future, results of the proposed studies might be exploited to increase semen fertility.

9702406 Uterine Chemokines
Hansen. T.R.; Perry, D.J. **Grant 97-35203-4808**

University of Wyoming
Department of Animal Science
Laramie, WY 82701

Strengthening Award
$130,000
3 Years

Pregnancy is dependent upon a receptive uterus which allows attachment and invasion of the conceptus while preventing immunological rejection. Uterine proteins (8-kDa) are secreted in response to interferon-tau (IFN-tau) during early pregnancy in the cow. Amino acid sequencing of four internal peptides revealed identity with the alpha chemokine family: bovine (b) granulocyte chemotactic protein-2 (GCP-2; 92-100% identity), and murine (m) macrophage inflammatory protein-2 (mMIP-2 (67-88% identity). Use of polyclonal bGCP-2 peptide antiserum in Western blot studies revealed that bGCP-2 is secreted by the endometrium during early pregnancy in response to conceptus-derived IFN-tau, and not during the estrous cycle or in response to the closely related IFN-alpha. Chemokines are potent chemo-attractants for cells of the immune system and have been implicated in inflammatory and angiogenic processes. We hypothesize that IFN-tau and the alpha chemokines alter secretion of endometrial cytokines to a humoral (T helper 2, TH2) rather than a cell-mediated (T helper l, TH1) phenotype. The chemokines may attract conceptus trophoblast and/or immunocytes to implantation sites where a directed immunostimulatory phenotype (TH2) coupled with scavenger effects (macrophage, neutrophils) would control inflammatory and angiogenic invasion of the maternal endometrium by the conceptus trophoblast.

Purified native uterine alpha chemokines and rbIFN-tau will be used to determine if they induce release of TH2 cytokines (i.e., interleukin (IL)-4 and IL-10) by cultured endometrial explants and peripheral blood lymphocytes. Alpha chemokines also will be tested for the ability to attract granulocytes, monocytes and trophoblast cells in a chemotactic bioassay. The bMIP-2 amino acid sequence is not known. Thus, uterine alpha chemokines will be examined further for amino acid sequence identity. The bGCP-2 peptide antiserum will be used to identify endometrial cells expressing the protein. The bGCP-2 cDNA will be isolated, sequenced and used as a probe to study transcription of bGCP-2 mRNA in endometrium during early pregnancy. Identification of the alpha chemokines in the uterine endometrium is novel. Also, the alpha chemokines represent the first novel markers of IFN-tau-specific action. The proposed experiments are the first to examine the IFN-tau/alpha chemokine system during early pregnancy.

9702434 Physiological Implications of Premature Induction of Ovulation
Murdoch, W.J. **Grant 97-35203-4611**

University of Wyoming
Department of Animal Science
Laramie, WY 82071

$130,000
3 Years

Hormonal control of ovulation is becoming a mainstay approach to manage reproductive efficiency. Success of early pregnancy is dependent upon ovulation of an ovum that can be transformed into an embryo capable of progressive development (intrinsic control) in a progestational oviductal/uterine environment (extrinsic control). Unfortunately, inappropriate presentation of a hormonal stimulus can lead to ovulation of an ovum that is not properly matured at the time of conception and (or) can result in the formation (from the ovulatory ovarian follicle) of a dysfunctional corpus luteum (i.e., progesterone deficiency). Remarkably little is know of the biological mechanisms that underscore the importance of ovum integrity and luteal phase sufficiency in the

establishment of pregnancy. Experiments will be performed in the sheep to evaluate the regulatory basis and physiological implications of the timing of ovulation induction (with gonadotropin-releasing hormone) on the embryogenic competence of the ovum and on congruent ovarian/oviductal/uterine functional activities. These studies will yield a fundamental understanding of how intrinsic and extrinsic factors interact to dictate pregnancy; and will be relevant in the design of ovulatory protocols that assure fertility in livestock.

IMPROVING ANIMAL GROWTH AND DEVELOPMENT

Panel Manager - Dr. Joan Burnside, University of Delaware
Program Director - Dr. Peter R. Brayton

Research in this program area contributes to our understanding of the biological mechanisms underlying growth and development in agriculturally important animals. Emphasis is placed on innovative approaches in several research areas including but not limited to: cell proliferation and differentiation, genetic mechanisms underlying growth and development, metabolic regulators such as growth factors, synthesis and degradation of protein and lipid at the cellular or tissue level, metabolic and nutritional aspects of growth and development including rumen microfloral development, developmental biology of the immune system, and cellular and molecular aspects of the effect of environmental stress on growth and development.

9703613 Transcriptional Regulation of Placental Hormones
Anthony, R.V. **Grant 97-35206-5092**

Colorado State University
Department of Physiology **$120,000**
Fort Collins, CO 80523-1683 **2 Years**

Sub-optimal fetal growth not only impacts the outcome of a given pregnancy, but also impacts postnatal growth and development. If a fetus develops slowly, it will exhibit a light birth weight, compromised neonatal survivability and postnatal growth rate. Conversely, if a fetus grows to fast, they will exhibit excess birth weight, leading to obstetrical complications, reduced neonatal survivability, and often to stunted postnatal growth rate. Regardless of the type of sub-optimal fetal growth, the placenta is a major determinant of the rate of fetal growth through its role in nutrient transfer and the production of placental hormones. One placental hormone, placental lactogen (PL), has been implicated as having a regulatory and/or modulatory role on fetal growth and metabolism. However, we know very little about how this placental hormone is regulated. We have isolated and characterized the gene encoding ovine PL (oPL), and have determined by *in vitro* assays that regions of DNA preceding the structural oPL gene likely regulate this gene in a tissue-specific manner. It is our objective to further define the responsible domains of DNA by *in vitro* methods, but more importantly to refine the location of the regulatory regions by the use of *in vivo* transgenic methodology. Defining the upstream DNA regions that regulate oPL gene function could provide insight into developing methods for modifying the production of this placental hormone to the advantage of mother and fetus. Furthermore, these regions of DNA could also be used to drive expression of desired products specifically within the placenta.

9703656 Endocrine Correlates and Nutritional Constraints on Environmental Adaptation and Growth in the Euryhaline Tilapia, *Oreochromis mossambicus*
Shepherd, B.S. **Grant 97-35206-5904**

University of Connecticut **Postdoctoral Fellowship**
Biotechnology Center **$90,000**
Storrs, CT 06269 **2 Years**

The Tilapia, *Oreochromis mossambicus*, which will be the focus of our studies, is an economically important finfish. The assent of tilapia aquaculture makes it inevitable that efforts will be directed toward the development of technologies to increase the growth of these fish to enhance production. Our objective is to develop a better understanding of how environmental salinity and nutrition interact to affect the hormones which regulate growth and adaptation in different environmental salinities. There are several metabolic processes in an animal that are necessary for adaptation and growth in an aquatic environment. Of the processes involved, the maintenance of salt and water balance (osmoregulation) is essential to life. To study osmoregulation one must also study growth since these two processes require considerable energy and are regulated by the same hormones (Growth Hormone: GH and Prolactin: PRL). The effects of GH on growth and of PRL on osmoregulation are well characterized in fishes; however, the effects of PRL on growth and of GH on osmoregulation are not. Our limited understanding of actions of GH and PRL in these processes is further complicated by findings that Insulin and the Insulin-like growth factors (IGF-I and -II) are involved in mediating some of the actions of GH and PRL, and that these intermediaries are themselves altered by environmental and nutritional factors. The aim of this study, therefore, is to clarify the roles that GH, PRL and their mediators (IGFs) play in the nutrition, growth and adaptation of tilapia to different environmental salinities.

9703612 Control of Functional and Cellular Losses During Bovine Mammary Gland Involution
Hurley, W.L. **Grant 97-35206-5098**

University of Illinois, Urbana-Champaign
Department of Animal Sciences
Urbana, IL 61801-3838
$120,000
2 Years

Milk secretion is directly related to the number of secretory cells in the mammary gland and to the lactation function of each cell. A key limitation to productivity of dairy cattle is the decrease in milk yield in the declining phase of lactation, which is associated with a loss of mammary cells and a loss of function of the cells remaining in the gland. Mammary involution induced by cessation of milk removal is an accelerated process similar to the gradual involution that occurs during the declining phase of lactation. Loss of milk secreting cells via programmed cell death occurs during involution, although the extent of cell loss in the bovine mammary gland is not clear. In addition, little is known about the factors which control induced involution. Concurrent pregnancy has a significant effect on milk yield persistency in late lactation and may be a major factor controlling rate of involution. This project will determine the role of pregnancy in controlling bovine mammary function during involution. It is hypothesized that concurrent pregnancy enhances the rate of morphological and functional changes which occur during involution in the dairy cow. Knowledge gained from understanding the loss of cells and tissue function during involution may lead to greater efficiency of milk production during the declining phase of lactation. This outcome would have a significant impact on the dairy industry and the consumer of milk and dairy products.

9703621 Molecular Analysis of Bacterial Community Structure and Function in the Rumen
White, B.A. **Grant 97-35206-5095**

University of Illinois
Department of Animal Sciences
Urbana, IL 61801
$223,974
3 Years

The long-term objectives of this research are to develop a molecular road map of the microbial community of the rumen, and to correlate populations with rumen function, by use of molecular methods to study phylogeny, microbial diversity and gene expression. Our initial model for correlating community structure to function focuses on bacteria involved in plant cell wall degradation. Our objective is to use molecular methods to critically analyze the key ruminal microorganisms involved in this process with respect to population density, diversity, and functionality. Gene libraries, nucleic acid probes and *in situ* hybridization will be used to provide a link between knowledge obtained in pure cultures and the microbial populations they represent in the rumen. The use of these tools will result in greater insights into community structure and activity of gut microbial ecosystems in relation to functional interactions between different bacteria, spatial and temporal relationships between different microorganisms and between microorganisms and feed particles. The successful development and application of these methods, promises to provide the first opportunity to link distribution and identity of gastrointestinal microbes in their natural environment with their genetic potential and in situ activities. The proposed studies are unique in that they combine approaches for assessing population dynamics with approaches for assessing community functionality. This will result in an increased understanding and a complete description of gastrointestinal community of production animals under different feeding regimes, and lead to new strategies for improving animal growth.

9703657 Regulation of Pituitary Development by the P-Lim Transcription Factor
Rhodes, S.J. **Grant 97-35206-5084**

Indiana University-Purdue University at Indianapolis
Department of Biology
Indianapolis, IN 46202-5132
New Investigator Award
$120,000
2 Years

The anterior pituitary gland contains specialized cells secreting hormones that regulate growth, lactation, reproductive development and status, thyroid physiology, and the stress response. During development, tissue-and cell-specific gene regulatory proteins coordinate the commitment and differentiation of the hormone-releasing cells. P-Lim is a pituitary-specific transcription factor essential for pituitary organogenesis: animals with mutated P-Lim genes do not develop anterior pituitary glands and die shortly after birth. The aim of this research is to characterize the mechanism of action of this critical protein in the pig. We will clone, sequence and characterize complementary DNA clones encoding pig P-Lim. To understand the transcriptional activity of P-Lim, the mechanism of pig pituitary target gene activation by P-Lim will be investigated. Further, to better understand hypothalamic and other signaling pathways that may regulate P-Lim function, modification of the pig P-Lim protein will be studied and the effects of such modification on P-Lim function determined. These studies will increase our understanding of essential developmental control mechanisms governing the central endocrine organ that coordinates growth, reproductive function and homeostasis in all animals of agricultural importance, including aquaculture species. This work will therefore guide future genetic and treatment protocols aimed at improving animal growth and fitness and will enable increased agricultural productivity.

9703930 Regulation of Leptin Gene Expression and Bioactivity in the Pig
Houseknecht, K.L. **Grant 97-35206-5093**

Purdue University **New Investigator Award**
Department of Animal Sciences **$110,000**
West Lafayette, IN 47907-1151 **2 Years**

Improving the productive efficiency of food animals requires understanding of the integrated biological processes involved in the regulation of adipose tissue metabolism, lean protein accretion and whole-body energy metabolism. Such processes are regulated by myriad hormones and growth factors which act to coordinate tissue nutrient utilization and food intake. The discovery of leptin, an adipostatin which senses and regulates body energy stores in rodents and humans, opens a new chapter in the understanding of energy metabolism. Circulating leptin levels in rodents and humans not only reflect body fat mass and nutritional status, but a hallmark of obesity is hyperleptinemia and leptin resistance. Data suggest that leptin may play a role in the development of peripheral insulin resistance associated with obesity. Leptin is also important in signaling insufficiency of adipose tissue stores during fasting or disease states. Our hypothesis is that leptin plays a key role in the regulation of feed intake and energy metabolism in the pig, and that regulation of leptin action may be important in optimizing animal growth performance and well-being. The first objective examines the role of fatty acids in regulating leptin expression *in vivo*. This includes quantifying adipocyte leptin expression in pigs fed isonitrogenous diets which vary in fat content (0-10%) and fatty acid profile (14:1/16: vs 18:2). The second objective involves identification of proteins in porcine serum which specifically bind leptin. The proportion of leptin in the free and bound state will be determined in fed and fasted, lean and obese pigs. Leptin binding proteins most likely play an important role in the regulation of leptin bioactivity and clearance rate.

9703998 Pig Leptin and Growth Hormone Secretion
Ramsay, T.G., Barb, C.R.; Steele, N.C. **Grant 97-35206-xxxx**

Louisiana State University
Pennington Biomedical Research Center **$190,000**
Baton Rouge, LA 70808 **3 Years**

The purpose of this project is to develop a novel method to elevate intake in young, growing swine, a period characterized by high rates of protein deposition and muscle protein accretion. Unfortunately, these rates are not maximized because young growing swine eat to satiety and not to gut fill. Elevating intake requires some knowledge of the mechanisms regulating feeding behavior in these pigs. Recently, our laboratory has identified the porcine variant of a protein (leptin) produced by adipose tissue that inhibits feed intake in growing pigs. This project is designed to assess the regulation and action of this protein on feeding behavior and growth of young pigs. The objectives of this project are: 1) identify the mechanism of action of growth hormone on leptin secretion; 2) determine how leptin regulates growth hormone secretion; 3) investigate the effect of leptin immunization on GH secretion, feed intake regulation and consequent protein accretion in growing pigs. Characterization of the proposed leptin/GH axis will provide new insights into mechanisms of body composition regulation. Implementation of this knowledge through the leptin immunization and GH supplementation experiments will permit us to develop strategies to maximize protein accretion in the young pig during the grower phase.

9704000 Behavior-induced Neural and Neuroendocrine Regulation of Pituitary Cell Function
Proudman, J.A.; Kuenzel, W.J. **Grant 97-35206-5087**

USDA, Agricultural Research Service
Germplasm and Gamete Physiology Laboratory **$140,000**
Beltsville, MD 20705-2350 **2 Years**

The pituitary gland hormones, which regulate many vital body functions, are each produced by specialized cells. It has been shown in mammals that pituitary cell function can change in the adult animal from production of one hormone (growth hormone) to production of a different hormone (prolactin) when prolactin is required for reproduction. This change occurs, at least in part, through an intermediate cell that contains both hormones. The turkey hen undergoes a dramatic increase in prolactin secretion during the change from egg-laying to incubation behavior that requires the stimulation of a neural pathway which involves both the peripheral and central nervous system. This tactile stimulation must be maintained, or a rapid reversal of pituitary cell function occurs and incubation behavior ceases. This research will study the hormonal and cellular mechanisms which cause adult pituitary cells to either differentiate, divide or change function in response to physiological and environmental signals, and how cell function is reversed when these signals are withdrawn. The turkey hen is advantageous for these studies since pituitary cells producing growth hormone and prolactin are anatomically separated within the turkey pituitary gland. The results may aid in understanding how cells change function in adult animals, and may provide information which will aid in the control of incubation behavior in the turkey. This behavior costs turkey producers $60 to $100 million per year in management expenses and lost hatching egg production.

9703618 Function of Butyrophilin in Lactation
Mather, I.H.; Mariuzza, R.A.; Linington, C. **Grant 97-35206-5085**

University of Maryland
Department of Animal and Avian Sciences
College Park, MD 20742-2311
$250,000
3 Years

Molecular mechanisms underlying the secretion of milk-fat droplets in the mammary gland will be studied with the long-term aim of reducing the levels of fat and cholesterol in milk. We will test the hypothesis that butyrophilin (BTN), the major protein of the milk- fat-globule membrane, is the principal structural component of a complex of proteins which interact with cellular fat droplets. Formation of this complex is postulated to promote binding to more hydrophobic proteins associated with the surface of intracellular fat droplets, which drives the expulsion of lipid from the cell. Recombinant proteins comprising specific domains of BTN will be produced and prospective interacting proteins identified by biochemical approaches. As a direct test of the possible function of BTN in lactation, the BTN gene will be ablated in mice and mammary gland development and lactation analyzed in the 'knock-out' mice. Recombinant proteins produced in this study will be used for an autoimmune study with the co-P.I., Dr. C. Linington. The proximal immunoglobulin domain of BTN is homologous to an immunoglobulin fold in myelin oligodendrocyte glycoprotein (MOG), a component on the surface of the myelin sheath. Exposure of rodents and primates to MOG elicits an autoimmune demyelinating response and an multiple-sclerosis-like pathology. We will test the hypothesis that because of molecular mimicry between MOG and BTN, dairy products consumed in the diet may elicit the inappropriate production of antibodies which cross-react with MOG and predispose individuals to autoimmune neurologic disease.

9706426 Secretory Function of Growth Hormone Cells during Chicken Development
Porter, T.E. **Grant 97-35206-5086**

University of Maryland
Department of Animal and Avian Sciences
College Park, MD 20742-2311
$250,000
3 Years

Strategies aimed at increasing growth rate and improving feed efficiency in chickens are extremely important to sustainable agriculture because feed is the largest cost of raising chickens for human consumption. Growth rate and feed consumption are controlled in part by growth hormone from the anterior pituitary gland. Better understanding of the contributions of growth hormone production during embryonic development to post-hatch growth of the chickens is essential to improve growth and feed efficiency through increasing naturally occurring growth hormone levels. The experiments in this proposal will continue our research into the endocrine regulation of growth in broiler chickens. We have determined that growth hormone cell differentiation during embryonic development is stimulated by a blood-borne signal, and we have identified this factor. Our preliminary findings indicate that administration of this compound during embryonic development can increase growth rate after hatching in broiler chickens. The overall goal of the research described in the current proposal is to improve growth characteristics in broiler chickens through inducing premature growth hormone cell differentiation during late embryonic development. Special emphasis will be placed on improving our ability to increase body weight gain and increasing our understanding of the mechanism underlying the effects of augmenting endogenous growth hormone production. The specific aims of this proposal are to: 1) characterize the artificially-induced growth hormone cell population; 2) define the mechanism underlying induced growth hormone cell differentiation; 3) Determine effects of *in ovo* treatment on post-hatch hormone secretion and growth. We plan to accomplish these objectives by measuring gene expression and hormone secretion with techniques currently in use in our laboratory. The results of these studies should provide information directly applicable to the poultry industry to increase poultry production for human consumption.

9703653 Molecular Biology of Protein Degradation and Utilization by *Prevotella ruminicola*
Morrison, M. **Grant 97-35206-5091**

University of Nebraska
Department of Animal Science
Lincoln, NE 68583-0908
$190,000
3 Years

As the management practices associated with meat and milk production continue to become more intensive, so too does the need to minimize nitrogen excretion in animal waste. Feeding protein-rich forages to ruminant animals often results in ammonium production at a rate which exceeds its utilization by the ruminal bacteria; as much as 25% of the protein-nitrogen fed to the animal may be excreted as a waste product. Any reduction in ruminal protein degradation, particularly in grazing and forage-fed livestock, may well have significant economic and environmental implications. Efficiency of animal growth and development will improve, and less nitrogen will be excreted into the environment. However, little mechanistic or quantitative information is available about specific enzymes thought to be relevant in controlling rates of ruminal protein degradation and ammonia production. The primary objectives for this proposal are: i) to clone and isolate the genes encoding the predominant proteolytic enzymes from *P. ruminicola*; ii) create proteinase defective strains of *P. ruminicola* and; iii) determine whether protein

degradation and ammonia production by predominant ruminal bacteria is altered. Such an examination should provide the resources necessary to determine whether and how highly selective means of *P. ruminicola* enzyme inhibition can be developed. Our ultimate objective is to provide the knowledge and resources necessary to identify new, highly selective means of proteinase enzyme inhibition, reduce the waste of protein, and decrease nitrogen loss to the environment.

9703931 Protein Restriction Stress and the Remodeling of the Immature Fowl Adrenal Gland
Carsia, R.V. **Grant 97-35206-5090**

University of Medicine & Dentistry of New Jersey
Department of Cell Biology
Stratford, NJ 08084-1489
$190,000
2 Years

In the domestic fowl adrenal gland, the balance of stimulating (tropic) hormones (adrenocorticotropic hormone, angiotensin II) not only finely regulates the secretion of adrenal steroids (corticosterone, aldosterone), but also influences the cellular composition (i.e., cellular remodeling) of the gland by regulating the components of remodeling: functional plasticity (i.e., ability to change secretion pattern), cell proliferation, differentiation and death (apoptosis). Certain stresses (e.g., nutritional stressors imposed during growth) alter the cellular composition and secretory setpoint of the gland. For example, dietary protein restriction induces cellular remodeling of the turkey adrenal gland and overall panhypofunction. By contrast, this same stressor induces adrenal growth and enhances adrenocortical cell function in the chicken, but it is not known if it causes cellular remodeling. Thus, these two commercial species provide a unique opportunity to determine how their adrenocortical cells differently integrate stressor information and interpret and segregate growth-, death- and steroid secretion-promoting signals. Young male chickens and turkeys will be subjected to low-protein diet for 4 weeks. Adrenocortical cell functional plasticity (i.e., corticosteroid responses to tropic hormones) and the signal-broadcasting properties of their tropic hormone receptors will be evaluated. In addition, the other components of remodeling, cell proliferation and apoptosis, will be evaluated. The significance of this work to the charge of the USDA is that it may lead to nutritional strategies to ameliorate abnormal remodeling and the concomitant deleterious secretion of corticosteroids and subsequent cardiovascular disease, suboptimal growth and development in response to environmental and physiological stressors.

9703625 Role of Parathyroid Hormone-Related Protein in Lactation and Neonatal Development
Rosol, T.J. **Grant 97-35206-5089**

Ohio State University
Department of Veterinary Biosciences
Columbus, OH 43210
$190,000
2 Years

Parathyroid hormone-related protein (PTHrP) is a newly discovered growth factor that is produced by the mammary gland and is present in the milk of all mammals (including processed, store-bought milk from cows, goats or sheep consumed by humans). The function of PTHrP is unknown, but it may play an important role in mammary gland development, milk production, calcium content of milk, and neonatal development. The overall goal of this proposal is to investigate the function of PTHrP in the mammary gland during lactation and in the suckling neonate. A transgenic mouse will be developed that has tissue-specific disruption ('knock-out') of the PTHrP gene in the lactating mammary gland. Tissue-specific disruption of the PTHrP gene is important since deletion of this gene from the entire animal results in death at birth due to abnormal bone development of the fetus. Disruption of the PTHrP gene only in the lactating mammary gland will enable studies on the role of PTHrP in the lactating gland and in the development of bones and the intestinal tract in suckling neonates since there will be no PTHrP present in the milk. Determining the function of PTHrP in the lactating mammary gland and neonate is crucial to increasing our understanding of mechanisms which regulate milk composition, lactation, and growth. These new and innovative research approaches will increase our knowledge of a major mammary gland hormone and improve our ability in the future to manipulate mammary function and the nutrient composition of milk.

9703619 Gordon Research Conference on Mammary Gland Biology
Schanbacher, F.L.; Neville, M.C. **Grant 97-35206-5083**

Ohio State University
Department of Animal Sciences
Wooster, OH 44691-9805
$20,000
1 Year

Support is requested for the Fourteenth Biennial Gordon Research Conference on Mammary Gland Biology held at Plymouth State College, Plymouth, NH, June 15-20, 1997, involving scientists from divergent disciplines with a common interest in mammary biology and lactation. The program, organized around research areas with rapid advances and to stimulate interdisciplinary advances in the field, includes sessions on: Questions in Mammary Biology and Disease; Breast Cancer Genes in Mammary Development and Neoplasia; Estrogens and Xenoestrogens in Mammary Development and Cancer; Prolactin and its Receptors in Mammary Regulation; Stromal and Autocrine Factors in Mammary Development; Transcription Factors and

Signal Transduction in Mammary Regulation; Pathways and Regulation of Milk Secretion; Bioactive Components of Milk; and Biochemistry and Molecular Regulation of Milk Proteins. Junior scientists (grad. students, postdoctoral fellows) will be encouraged through poster session highlights presentations and participation in a workshop on Mammary Specific Gene Deletion. To encourage new scientists into the field, 21 of the 28 invited speakers have not spoken previously on the program of this Conference. Interdisciplinary discussion of ideas and information between dairy and animal scientists and basic biomedical researchers is encouraged by conference presentations and discussions which will not be published or cited, the discussion-intensive program, and an informal atmosphere. Several presentations relate to agricultural mammary and lactation biology and target areas for the Research Initiative in Improving Animal Growth and Development. Funding is requested in support of selected invited speakers whose topics fit interests of the USDA and agricultural mammary scientists, and to facilitate participation of junior scientists.

9703588 A Molecular Approach to Enhance Muscle Protein Accretion: Proof-of-concept
Forsberg, N.E. **Grant 97-35206-5097**

Oregon State University
Department of Animal Science **$245,000**
Corvallis, OR 97331-6702 **3 Years**

The overall goal of this research program is to develop a strategy to increase muscle growth in domestic animals. To accomplish this, however, we must first identify the constraints to growth in animals and develop a strategy to effectively manipulate these constraints. Our previous research has indicated that calpain (calcium-activated neutral protease) activity slows muscle growth. Hence, the goal of this work is to develop a genetic strategy to manipulate muscle growth in living animals, to evaluate the effects of manipulating calpain activity in cultured muscle cells and, finally, to assess the impact of manipulating the activity of the calpains *in vivo* on rates of muscle growth. To accomplish the first goal, we will modify the ecdysone promoter system so that it will function in skeletal muscle only. In the second phase of the research, we will study the utility of this muscle promoter system in cultured muscle cells and, in this study, assess the impact of regulating one calpain isoforms (μ-calpain) on myofibrillar protein stability. In the final study, we plan to prepare a transgenic mouse in which we may manipulate calpain activity in vivo and thereby assess calpain function and the utility of controlling calpains as a strategy to enhance growth. This research represents an ambitious attempt to develop strategies to increase muscle growth and will also reveal important roles of calpains in muscle of living animals.

9703648 Dietary Manipulation of Metabolism in Salmon Smolts
Tremblay, G.C. **Grant 97-35206-5288**

University of Rhode Island **Strengthening Award**
Department of Biochemistry, Microbiology and Molecular Genetics **$120,000**
Kingston, Rl 02881-0000 **2 Years**

Juvenile salmon must undergo major metabolic changes before they are able to migrate from the freshwater streams, where they were hatched, to the open ocean, where they grow to maturity. This salt-water challenge is gradual in nature, but it is quite abrupt in commercial aquaculture, where salmon are transferred directly from freshwater hatcheries to sea water netpens. Salt-water tolerance requires considerable expenditure of energy, yet transfer occurs at a time in the life cycle of salmon when carbohydrate-energy reserves in the liver are virtually depleted. Losses upon transfer to sea water can be high. There are specific nutrients known to stimulate deposition of carbohydrate-energy reserves in the liver, and we propose to feed such nutrients to juvenile Atlantic salmon for several months prior to sea water transfer. We plan to determine whether feeding these dietary additives prevents depletion of carbohydrate-energy reserves in the liver, and whether such action is associated with biochemical evidence of improved salt water tolerance. We will also determine whether salmon fed such diets exhibit greater sea water survival and growth.

9704035 Postnatal Involution of Brown Fat in *Bos taurus* and *Bos indicus* Calves
Smith, S.B.; Carstens, G.E. **Grant 97-35206-5134**

Texas A&M University **New Investigator Award**
Department of Animal Science **$100,000**
College Station, TX 77843-2471 **2 Years**

Suboptimal growth and development are limiting factors in animal productivity, and neonatal calf mortality losses represent a major biological restraint to beef cattle production. In Texas alone, current estimates indicate that approximately 432,000 calves die annually during the neonatal period, which results in revenue losses of more than $200 million. This is particularly significant for beef producers in Texas, since more than half of the 5.4 million beef cows in Texas are Brahman or Brahman crossbred cattle. *Bos indicus* cattle are more susceptible to neonatal losses than *Bos taurus* cattle. Brown adipose tissue (BAT) thermogenesis is an important component for proper thermoregulation during the early neonatal period. Although our knowledge of the

mechanisms controlling BAT thermogenic function in laboratory animals has advanced greatly in recent years, much less is known about these regulatory mechanisms in ruminant neonates. We propose and will test the following hypotheses regarding possible mechanisms that contribute to dysfunctional BAT thermogenesis in Brahman calves. First, thermogenic capacity, *beta*-adrenergic receptor populations, and UP gene expression will decline more rapidly early postnatally in Brahman calves than in Angus calves, especially in response to cold exposure. Second, Brahman BAT will exhibit lesser capacity to synthesize and, especially, oxidize fatty acids in vitro than Angus BAT early postnatally and in response to cold exposure. This also will be reflected in the morphological characteristics of the BAT depots. This research will provide specific information on the regulation of lipid turnover and adipocyte differentiation at the cellular level. This proposal also is a carefully designed investigation of the cellular and molecular aspects of the effects of the environment (*i.e.*, cold vs. warm exposure) on adipose tissue growth and development. Elucidation of these regulatory mechanisms eventually will lead to the development of strategies to enhance BAT function, thereby improving cold tolerance and survivability of newborn calves during inclement weather.

9704036 Arginine Metabolism in Enterocytes of Developing Pigs
Wu, G. **Grant 97-35206-5096**

Texas A&M University
Department of Animal Science
College Station, TX 77843-2471

$160,000
2 Years

Enterocytes (epithelial absorptive cells of the small intestine) are not only responsible for absorption of nutrients, but they also actively participate in metabolism of amino acids (nutrients) Early weaning of piglets, although increasing the sow's productivity, causes intestinal atrophy, malabsorption of nutrients and decreased growth, a major problem in the swine industry. Glutamine arginine and proline are essential amino acids for piglets and play an important role in intestinal morphology and function. Our overall goals are to elucidate the mechanism for the regulatory role of amino acids in intestinal growth and development of postnatal pigs. The specific objectives of this project are to quantify proline metabolism and arginine synthesis from proline in enterocytes of post-weaning pigs; to determine changes in messenger ribonucleic acid (mRNA) levels for intestinal arginine-synthesizing enzymes in postweaning pigs compared with age-matched suckling pigs; to determine the role for cortisol surge in regulating the mRNA levels for intestinal arginine-synthesizing enzymes in post-weaning pigs; and to determine whether cortisol plays a role in regulating proline metabolism and maintaining intestinal morphology in post-weaning pigs. Results of this study will establish a novel pathway for the synthesis of citrulline and arginine from proline in pig enterocytes. Our findings will help to elucidate the cellular and molecular mechanism for the cortisol regulation of arginine metabolism in pig enterocytes. A better understanding of arginine metabolism in pig enterocytes is essential to designing means to prevent intestinal atrophy and improve growth performance in early-weaned pigs.

9703641 Modulation of Porcine Adipocyte Hyperplasia and Differentiation by Fatty Acids
Mersmann, H.J. **Grant 97-35206-5133**

USDA Agricultural Research Service
Children's Nutrition Research Center
Houston, TX 77030-2600

$120,000
2 Years

Fat is added to diets for pigs, poultry, and cattle to reduce dust, as an energy source, to stimulate appetite, and to make meat product fatty acid (FA) composition more favorable for human health. Individual FA from dietary fat are incorporated into most cells, both in storage fat globules and as an integral part of various cellular membranes. The FA composition of membranes determines the fluidity of membranes and the function of some membrane-bound proteins. Adipocyte lipid metabolism pathways may be altered by some FA with increased fat deposition. Some adipocyte genes are controlled by FA. The many-fold biological effects of individual FA make it important to understand not only gross effects of added dietary fat but the biological implications of individual FA on the differentiation and growth of the adipocyte. Individual FA vary greatly in carbon chain length, degree of unsaturation, and biological effects. To sort these effects, porcine adipocyte growth and differentiation will be studied in cell culture. Undifferentiated precursor cells will be isolated, grown, and differentiated in the presence of individual FA. Cell number, differentiation, the messages for genes that indicate differentiation (lipoprotein lipase) and genes for receptors (insulin and *beta*-adrenergic) that control adipocyte lipid metabolism, enzyme activity, receptor affinity, and adipocyte and membrane FA composition will be quantified. Results will provide a data base to select a dietary FA composition to optimize not only the cost and gross animal growth effects but also the equally important growth and function of the adipocyte.

IDENTIFYING ANIMAL GENETIC MECHANISMS AND GENE MAPPING

Panel Manager - Dr. Charles F. Louis, Univeristy of Minnesota, St. Paul
Program Director - Dr. Peter R. Brayton

The objective of this program is to increase our knowledge and understanding of the structure, organization, function, regulation and expression of genes in agriculturally important animals including aquaculture species. This includes but is not limited to: gene mapping and the identification, isolation, characterization of genes, gene products and their regulatory mechanisms, identification and mapping of DNA segregation markers including quantitative trait loci (QTL) and variable number tandem repeats (VNTR), interactions between nuclear and organellar genes and the molecular basis of genetic replication, and development and application of methods to modify the animal genome.

9703983 Proliferation and Differentiation of Cultured Porcine Primordial Germ Cells
Anderson, G.B. **Grant 97-35205-5076**

University of California, Davis
Department of Animal Science **$200,000**
Davis, CA 95616-8521 **2 Years**

Opportunities are rapidly becoming available to augment traditional animal breeding strategies with new technologies that allow manipulation of specific genes. These new technologies involve making genetic modifications in cultured cell lines in the laboratory, and then incorporating the modified cells into a viable embryo for development to term. Appropriate cell lines are available for this purpose in the laboratory mouse, and they are being used extensively to create unique genetic models for biomedical research. Unfortunately, cell lines for use to add, replace or delete a specific gene are not available in large animals of agricultural importance. We propose to develop such cells lines for use in the pig. In a previous USDA-funded project, we demonstrated that cells called primordial germ cells can be isolated from 25-day-old porcine embryos and maintained in the laboratory. The cells were demonstrated to retain the capacity to develop into normal tissues, in particular, into somatic tissues (all tissues except gametes). We did not demonstrate development into gametes (*i.e.*, eggs and sperm), which is essential for use of the cell lines for genetic modification. Our current project is aimed at testing several methods of achieving normal development of our porcine cell lines into normal tissues, including gametes. The first series of experiments will involve study of development during early pregnancy. Subsequent experiments will include development to term and examination of the resulting piglets for evidence that our cell lines can develop into gametes.

9703597 Multipoint Mapping of QTL and Marker Assisted Selection in Outbred Populations
Xu, S. Z. **Grant 97-35205-5075**

University of California, Riverside
Department of Botany and Plant Sciences **$140,000**
Riverside, CA 92521-0124 **3 Years**

Traits that appear to be continuously distributed are called quantitative traits, *e.g.*, body weight and milk production. The expression of a quantitative trait is partially controlled by heritable factors (genes) and partially determined by random environmental factors. Genes that control quantitative traits are referred to as quantitative trait loci (QTL). Identification and localization of these QTL (also called QTL mapping) are important in understanding the genetic mechanisms of quantitative traits and developing efficient selection programs for genetic improvement. Traditional methods of QTL mapping require special breeding programs such as inbreeding and cross breeding. Because of this, they are applicable only in heavily manipulated situations such as with agricultural crops or laboratory animals. As-of-yet, there is no general method to map QTL in arbitrarily bred populations such as beef cattle. This research will address this deficiency and present efficient methods that are not only applicable to arbitrarily bred populations, but are both statistically more powerful and address a wider inference space. One objective of the proposed research is to develop a general multipoint method for QTL mapping and marker assisted selection in outbred populations. By multipoint, we mean that all markers, including fully informative and partially informative markers are used simultaneously to infer the probabilities of allelic transmission of candidate QTL. The second objective of the project is to develop a cost efficient marker assisted two-stage selection scheme applied to outbred populations. Finally, we plan to release a package of computer programs for QTL analysis and marker assisted selection using the algorithms proposed in this research.

9703929 Sequence of Trophectoderm RNA Expression Throughout Porcine Conceptus Elongation
Ford, S.P.; Smith, T.P.L. **Grant 97-35205-5077**

Iowa State University
Department of Animal Science
Ames, IA 50011-3150
$150,000
3 Years

On day 11-12 of gestation, pig embryos undergo a rapid (12-24 hours) change in shape from a 9-10 mm sphere to a one meter long thread known as elongation. We hypothesize that the length attained by a porcine conceptus at elongation dictates placental size which in turn affects the number of embryos carried to term. This hypothesis is supported by the results of recently completed experiments utilizing the prolific Chinese (Meishan) pig which averages 3 to 5 more pigs per litter than U.S. pig breeds in spite of an ovulation rate and a uterine size similar to U.S. pig breeds. This increase is accomplished by a marked reduction in placental size, reducing the area of uterus occupied by each conceptus. We have recently reported that the trophectoderm cells of Meishan embryos exhibit a reduced mitotic rate throughout pre-implantation development when compared with Yorkshire embryos. Further, Meishan embryos elongate with fewer trophectoderm cells and are smaller than Yorkshire embryos on day 14. Elongation of the most developed embryos in a litter is associated with the death of lesser developed litter mates as a result of as yet undefined changes in the uterine environment. In spite of the extreme importance of this event to the establishment of pregnancy in the pig, the pattern of gene expression during elongation has not been identified. We propose to begin to identify and map genes expressed in embryonic tissue immediately before, during and after elongation. A further comparison of genes so identified in the Yorkshire with Meishan embryos of similar stages will be done.

9703610 Towards an Integrated Genetic and Physical Map of the Bovine Genome
Lewin, H.A.; Ma, R.Z. **Grant 97-35205-4738**

University of Illinois, Urbana-Champaign
Department of Animal Sciences
Urbana, IL 61801-3838
$275,000
2 Years

The development of a genetic linkage map of the bovine genome was a primary research goal of the National Animal Genome Research Program. This early goal has been realized and exceeded. Within the scientific community it is now generally accepted that a high resolution linkage map and a physical map of regions where genes controlling economically important traits reside are required to maximize the potential of the overall genome effort. The integration of an accurate, high resolution linkage map with the physical map of the bovine genome will permit the identification of genes that influence economically important traits. For this research, we will construct an ordered physical map of bovine chromosome 23, a region that contains genes important for immune responses, growth and development. We will also physically map an interval that contains sequences responsible for creating new genetic variation by recombination. The proposed research will thus contribute to an integrated, high resolution genetic and physical map of the bovine genome. Application of the advanced mapping strategies used in this research will also provide a foundation for the completion of an ordered map of cattle genes. Realization of the long-term objectives of this research will enhance the competitiveness of U.S. dairy and beef industries in national and international markets via improved efficiency of production systems and yield of quality products.

9703996 Identification and evaluation of the acid meat (RN) gene in swine
Sunden, S.L.F. **Grant 97-35205-5078**

University of Illinois, Urbana
Department of Animal Sciences
Urbana, IL 61801-3838
New Investigator Award
$290,000
3 Years

Meat quality is an issue of increasing importance to animal agriculture and to the swine industry in particular. A recent US industry survey has shown that meat quality defects are a source of major economic loss to the swine industry. One quality issue of major concern in the US is the so-called "acid meat condition". This condition, as the name implies, results from a low ultimate pH in the muscle post mortem. Compared to normal pork, acid meat is paler and has lower water holding capacity, which results in increased cooking loss and a reduction in processing yields. The net result is a negative impact on the financial return to the meat processor. On the other hand, acid meat has been shown to be more tender and the condition may be associated with enhanced growth and carcass characteristics. Thus, there is a potential advantage that may be of economic significance in certain situations. The goals of this project are to utilize a constellation of expertise and resources to identify the gene that causes the RN phenotype and characterize its effects on meat quality and production traits.

9703789 Genetics of Between-breed Resistance to Nematode Infection in Sheep
Miller, J.E.; McGraw, R.A. **Grant 97-35205-5081**

Louisiana State University, Baton Rouge
Department of Epidemiology and Community Health
Baton Rouge, LA 70803-8404

$262,587
3 Years

Rural economics depend heavily on the viability of the family farm and most are involved in beef and forage production. Families that would like to start or return to such farming cannot generate the capital investments required. Small ruminants provide a viable alternative animal enterprise. Development of such enterprises in the Southeast, where a majority of U.S. forage is grown, is hampered by a serious gastrointestinal worm parasite constraint. Control traditionally relies on deworming with drugs. Sheep worm parasites have readily developed resistance to most dewormers available in the United States due to overuse. Environmental contamination concerns and consumer pressure seeks to restrict drug usage and reduce residues in meat and meat products of all livestock. Another approach to control is using genetically resistant animals. We have demonstrated that the Gulf Coast Native breed is more resistant to worm infection than the Suffolk breed. The long-term goal of our research program is to determine the reasons for this difference and to identify genetic markers for selection purposes. The objectives of this study are to evaluate segregation of resistance to worm infection using an F2 reference family approach, to identify linked genetic markers with resistance, and to map the resistance trait location on the sheep gene map using such markers. At the conclusion of this study, marker-assisted selection for resistance to worm infection may be possible in commercial sheep production systems worldwide, with potential applications to other livestock species.

9703919 International Nomenclature Workshop
Davisson, M.T. **Grant 97-35205-5080**

The Jackson Laboratory
Bar Harbor, ME 04609-1500

$5,000
1 Year

The rapid growth of comparative genome mapping and the integration of biological information derived from various model organisms have become powerful tools in understanding the structure and function of species genomes. Accurate terminology is essential for effective communication both in the literature and in database cross-linking in order to expedite publications by the scientific community in an understandable and consistent manner. The International Nomenclature Workshop, held at The Jackson Laboratory from April 30 through May 3, 1997, served as a forum for discussing current nomenclature issues and for generating ideas for dealing with the rapid growth in our knowledge of gene information and the coordination of this information among nomenclature groups for different species. Although the mouse and human nomenclature committees have met formally in the past, this was the first multi-organism genetic nomenclature workshop. The workshop brought together approximately 40 scientists representing various species (vertebrates, invertebrates, yeast and plants), and databases (MGD,GDB,GSDB,EMBL,NCBI) to discuss nomenclature issues such as 1) developing systematic approaches to nomenclature and symbol assignment across the species, 2) improving links between databases, 3) organizing genes into gene families and retrieving family information from databases, 4) fostering working relationships among those involved in nomenclature, and 5) encouraging community curation of gene family designations across species. Genome databases are being developed within the USDA National Animal Genome Research Program for four major groups, cattle sheep, pigs and chickens, with plans for development for horses and some aquatic species. Nomenclature guidelines are essential to the development of these databases because nomenclature is at the core of the growing effort to integrate biological information from various organisms. A summary manuscript of the Workshop is being prepared by the organizing committee for publication in Genomics.

9703976 Transplantation and Reprogramming of Somatic Nuclei in the Bovine
Jerry, D. J.; Robl, J. **Grant 97-35205-5132**

University of Massachusetts, Amherst
Department of Veterinary and Animal Sciences
Amherst, MA 01003

$172,000
2 Years

The goal of this project is to develop nuclear transfer procedures that will allow the production of large numbers of genetically identical cattle and allow the efficient production of transgenic cattle bearing precise alterations in the genome. Somatic cells from calves or adult cattle offer ideal sources of nuclei because they are readily available. Furthermore, nuclei donors could be selected based on proven genetic merit. However, preliminary data suggest a much poorer development of nuclear transfer fetuses when donor nuclei are from adult fibroblasts compared to embryonic fibroblasts. The experiments will document this preliminary result in greater detail with careful consideration of possible differences in the cell cycle distribution in these two populations of fibroblasts. One reason for the greater failure using adult fibroblasts for donor nuclei may result from an inability to undergo transcriptional silencing that occurs normally following nuclear transplantation. A transgene that produces a green fluorescent protein (eGFP) will be used to assess the fidelity of transcriptional silencing in embryos resulting from nuclear transplantation of adult and embryonic fibroblasts.

9703977 Immunoglobulin gene diversification and B cell development in cattle
Osborne, B.A. **Grant 97-35205-5072**

University of Massachusetts, Amherst
Department of Veterinary & Animal Sciences
Amherst, MA 01003
$330,000
3 Years

An effective immune response in all vertebrates is dependent, in part, upon the production of antibodies or immunoglobulins that specifically recognize the pathogen that initiates this response. In order to adequately recognize and neutralize the numerous pathogens encountered in a lifetime, it is necessary to diversify the repertoire of immunoglobulins produced. The mechanisms by which this diversity is achieved varies amongst mammals. In particular, the mechanisms by which cattle generate immunoglobulin diversity is not well understood. This project seeks to delineate this important process. Additionally, it is known in mouse and human how and where B lymphocytes develop into functional cells capable of synthesizing and secreting immunoglobulin. This process is not understood in cattle and there is ample evidence to suggest B cell development may be rather unique in the Bovidae. Thus another goal of this project is to understand the processes that lead to the development of functional B cells in cattle. It is expected that these studies will provide a framework of information that will allow manipulation of immune responses in cattle.

9703591, Chicken Genome Mapping Using AFLP and Anchor STS Markers
Dodgson, J.B. **Grant 97-35205-5131**

Michigan State University
Department of Microbiology
East Lansing, MI 48824-1101
$180,000
2 Years

Domestic breeds now utilized in animal agriculture are the product of hundreds of years of genetic enhancement by selective breeding. New technology that directly measures variation in DNA allows one to efficiently develop genome maps for domestic species. These maps help to explain how traits of interest (like disease resistance) are inherited and can be used in breeding. Our project is designed to improve the genome map of the domestic chicken by employing a new type of automated genetic marker system, called AFLP, that to date has been used mainly in plant species. If successful, this should allow traits of economic value to be mapped more quickly and in a wider variety of commercial poultry lines. We also are adding genes to the chicken map that are of known function and which have already been positioned on the human (or mouse) map. This strategy will allow the tremendous amount of basic knowledge now being generated about human (and mouse) genes to be applied to the genetic improvement of poultry. Overall, we hope to make it easier for breeders to design and implement efficient strategies to enhance the quality, safety and cost-efficiency of domestic poultry products.

9703922 Verification and refinement of QTL for carcass traits on swine chromosome 6
Miller, L.M. **Grant 97-35205-5079**

University of Minnesota, St. Paul
Department Veterinary PathoBiology
St. Paul, MN 55108-6010
Postdoctoral Fellowship
$90,000
2 Years

This project proposes to verify and refine the position of putative genes, called quantitative trait loci (QTL), responsible for carcass traits distinct from a disease causing gene (*ryr1*) on swine chromosome 6. It has long been recognized that numerous lean carcass characteristics are associated with the Porcine Stress Syndrome (PSS) causing allele at this locus. Because of this linkage, selection for leanness concomitantly increases the incidence of PSS which results in increased mortality and inferior meat quality. It is therefore important to better define the chromosomal region responsible for superior carcass qualities in order to improve these traits independently of the *ryr1* locus. A previous QTL scan of the Illinois reference family (Meishan X Yorkshire) identified a possible QTL for loin eye area positioned between markers adjacent to *ryr1*. Newly developed and existing markers will be genotyped on this family and included in a second analysis to refine the QTL position and effect. Chromosome 6 markers will then be genotyped on an additional resource population to evaluate their usefulness in identifying QTL in this population. This project should identify markers appropriate for use in marker assisted selection to improve this economically important trait in commercial swine herds.

9704290 Fine mapping of putative QTLs affecting ovulation rate on porcine chromosome 8
Alexander, L.J.; Schook, L.B.; Beattie, C.W. **Grant 97-35205-5082**

University of Minnesota, St. Paul
Department of Veterinary PathoBiology **$200,000**
St. Paul, MN 55108 **2 Years**

Ongoing research efforts are developing tools and reagents to identify areas of pig chromosomes which encodes genes controlling growth, carcass and reproductive traits. A large full-sib family was bred at the University of Illinois between Yorkshire and Meishan (a Chinese breed) pigs. Yorkshire pigs were chosen because of their excellent growth and carcass characteristics while Meishan were chosen because of their excellent reproductive traits. Thus the offspring will have genes controlling these economically important traits. By creating a three generation family, researchers can follow the segregation of chromosomal regions with various traits. Over 75 traits were recorded on approximately 300 offspring. DNA was obtained from each individual animal and through genetic analysis the origin of each (Yorkshire or Meishan) for each chromosomal region is being determined. One of the findings was an association of a region on pig chromosome 8 with an increased number of corpora lutea. Corpora lutea are the structures on the ovaries that expel ova during ovulation. Hence an increase in the number of corpora lutea increases the number of ova available for fertilization which translates into an increase in litter size. In order to refine the study of this region of pig chromosome 8 associated with increased corpora lutea we need to develop more reagents, i.e. genetic markers, to study this effect in our population and other populations and breeds. Thus the markers produced by this study can be used to directly select animals, via marker assisted selection, to eventually increase litter size and hence allow producers to be more competitive in global markets.

9703982 Organization and Expression of Genes in the Bovine Major Histocompatibility Complex
Skow, L.C. **Grant 97-35205-5074**

Texas A&M University
Department of Veterinary Anatomy and Public Health **$200,000**
College Station, TX 77843-4458 **3 Years**

Genes within the Major Histocompatibility Complex (MHC) have been associated with predisposition to a large number of disease conditions in humans and other mammalian species. Similar genetic components contributing to disease susceptibility occur in the MHC of cattle (BoLA) but are difficult to localize due to the lack of knowledge concerning the genetic organization of BoLA. The goal of this research is to define the arrangement, structure and function of genes within the bovine MHC in order to relate the genetic organization and content of BoLA to the MHCs of other species. Analysis emphasizes the class I loci. Results of this study will be an improved understanding of the genetic map of BoLA in a way that allows extrapolation of genetic information from other species, especially human and mouse, to improve cattle health and productivity.

9703984 Radiation Hybrid Mapping of the Bovine Genome
Womack, J.E. **Grant 97-35205-5073**

Texas A&M University
Department of Veterinary Pathobiology **$190,000**
College Station, TX 77843-4467 **3 Years**

Recent advances in bovine genome research have provided the tools for mapping economically important traits to specific regions of cattle chromosomes. Researchers have successfully applied these resources to mapping several traits of importance to the dairy and beef industries and many more traits will be mapped in the next decade. Unfortunately, the tools are not available for the next important step which is identifying and isolating the actual genes responsible for these traits. Important components of the human gene hunt are radiation hybrid (RH) maps. These maps provide the framework for building high density maps of gene transcripts necessary for candidate positional cloning of mapped genes. RH maps do not exist for livestock species. In addition to providing a framework for bovine transcript mapping, a bovine RH map will also facilitate high resolution comparative mapping, presenting candidate genes from human maps for cattle traits mapped to conserved regions of bovine chromosomes. We propose to generate a whole genome panel of 90 clones for bovine RH mapping. This 5000 rad panel will be used to generate framework maps for bovine chromosome with anonymous markers from existing linkage maps interspersed with conserved genes for comparative mapping. High density maps of targeted regions and transcript maps will be generated in our laboratory and through collaborations. Sufficient DNA will be generated from this panel to serve the mapping community well into the next century, providing a resource that will help break the current stalemate in the journey from mapped traits to isolation of the responsible genes.

9703988 Identification of the Defective Gene Responsible for Spider Lamb Syndrome in Sheep
Cockett, N.E. **Grant 97-35205-5129**

Utah State University
Department of Animal, Dairy and Veterinary Sciences
Logan, UT 84322-4700

$175,000
2 Years

Spider Lamb Syndrome (SLS) is a congenital disorder in sheep causing severe skeletal abnormalities such as abnormally long, spider-like legs, twisted spines, and deformed sternebra. The syndrome was first identified in black-faced lambs during the mid-1970's and has surfaced in several sheep breeds within the last two decades, including North American Suffolks and Hampshires, and U.S. Southdowns and Shropshires. While dramatic culling of all suspected carriers would reduce the frequency of the gene, it is a long and very expensive process. Progeny testing of potential breeding rams is another method of reducing gene frequency but it is also costly. Because of the drawbacks of traditional animal breeding methods, a genetic marker for Spider Lamb Syndrome will be an important tool for eliminating the SLS defect from sheep populations. Recently, genetic linkage between several genetic markers and SLS has been identified, thereby mapping the SLS locus to the end of ovine chromosome 6. Comparisons of ovine chromosome 6 to its evolutionary human and mouse homologues revealed a positional candidate gene, fibroblast growth factor receptor 3 (FGFR3). Preliminary results identified a polymorphism in the ovine FGFR3 gene that segregated perfectly with the SLS defect. However, to confirm that a defect in the FGFR3 gene is responsible for SLS, several experiments are required. In this project, we will determine differences in the FGFR3 gene of normal and Spider animals and then test these differences for association with the SLS defect in several populations of sheep. In this way, the causal mutation responsible for SLS will be identified.

9703915 Analysis of Somatic Mutations Induced by Specific Immune Responses in Trout
Kaattari, S. L. **Grant 97-35205-5130**

College of William and Mary Virginia Institute of Marine Science
Department of Environmental Sciences
Gloucester Point, VA 23062-1346

Strengthening Award
$162,000
2 Years

The exquisite specificity and affinity of antibodies for invading pathogens is essential to the induction of protective immunity. An essential element in the acquisition of these high affinity antibodies is the process of somatic mutation. During an immune response, mutated antibody-producing cells which possess the highest affinity for the pathogen become predominant among the responding lymphocytes, resulting in a shift to the production of more highly specific antibodies. This process of somatic mutation is well-characterized in mammals, but has only been observed recently in fish. Thus far these few reports in fish have not addressed the role of somatic mutation in the development of specific immune responses. It is the purpose of this study to examine the role of somatic mutation in the development and maturation of the protective antibody response to the salmonid pathogen, Infectious Hematopoietic Necrosis Virus (IHNV). Our studies will be focusing on the antibody response to the surface glycoprotein (G) of IHNV, which can confer protective immunity. Past work in mammals indicates that somatic mutations occur in response to protein antigens. Thus, this study uses a protein antigen, not only as a general immunological model protein, but one which has direct application to disease resistance in trout. It is anticipated that we may be able to derive knowledge of the conditions that are essential to the formation of the highest affinity antibodies to viral pathogens, and use this knowledge to develop more efficacious vaccine methodologies.

9703596 Gene Transfer in Chicken Bursal Stem Cells
Neiman, P. E. **Grant 97-35205-5159**

Fred Hutchinson Cancer Research Center
Division of Basic Science
Seattle, WA 98109-1024

$150,000
2 Years

The immune system has two basic types of cells, B and T cells. The B-cell immune compartment, is composed of cells which manufacture and secrete antibodies used in the body's defenses against germs and other foreign invaders. In chickens and other birds the B-cell compartment develops in a special organ called the bursa of Fabricius. This laboratory has developed a gene-transfer technique in which engineered genes can be introduced into bursal stem cells which are then transplanted into recipient birds whose bursal cells have been selectively destroyed by drug treatment. The gene-modified bursal cells migrate to and populate the bursa and the B-cell immune compartment in the recipient birds. We plan further to refine and exploit this technology in order to test the effects of engineered genes on B-cell development in the bursas of recipient birds, principally genes expressing antibody molecules from different stages of development. Chickens concentrate very large amounts of antibody in egg yolk. We plan to see if our engineered antibodies will also concentrate in egg yolk. If so, by gene splicing, we will attach novel proteins to our antibody genes and determine if we can use this system to concentrate any protein we chose in egg yolk. In this manner chicken eggs could be the source of large quantities of easily purified protein for commercial, nutritional, medical or scientific use.

SUSTAINING ANIMAL HEALTH AND WELL-BEING

Panel Managers - Dr. Carole Bolin, USDA-ARS-NADC,
Dr. Allison R. Ficht, Texas A & M University, Dr. Robin W. Morgan, University of Delaware
Program Directors - Dr. Peter J. Johnson, Dr. Peter R. Brayton

The objectives of this program are to increase the knowledge needed to sustain animal health and well-being and to prevent or reduce the severity of animal disease. This includes, but is not limited to: mechanisms that alter the normal physiologic state at the molecular, cellular or organ level to produce disease resulting from either biotic or abiotic causes; cellular mechanisms of disease resistance, including developmental and molecular immunology; microbial genetics; pathogenesis; both host and microbial factors influencing colonization of mucosal surfaces; host-environment or host-agent interactions that compromise host defense systems or cause predisposition to disease; epidemiologic studies on animal diseases that provide insight into etiologic factors and/or disease control; research that supports the development or evaluation of diagnostic tests and immunizations for emerging or reemerging disease problems such as tuberculosis; studies on economic models that address the costs of animal disease and the cost/benefit ratios of animal disease prevention and therapy. The program also encourages research on the mechanisms controlling animal responses to physical and biological stresses (including quantitative behavioral, physiological, immunological and neurobiological responses to stress) and the development of objective indicators to measure animal well-being.

9702311 Functional Analysis of MDV pp24/pp38-Encoding Genes
Parcells, M.S. **Grant 97-35204-5067**

University of Arkansas, Fayetteville **Strengthening Award**
Department of Poultry Science **$185,000**
Fayetteville, AR 72701-1201 **3 Years**

Marek's disease (MD) is a cancer of chickens caused by Marek's disease virus (MDV), a cell-associated herpes virus. MD is a major concern to the poultry industry because tumor-bearing chickens are condemned at processing. MDV-induced tumors are comprised of transformed T-lymphocytes. How MDV transforms these T-cells is currently unknown, but studies examining what MDV genes are active in the tumors and cell-lines established from these tumors have identified a number of potential oncogenes. Two such genes encode phosphorylated proteins pp24 and pp38. The genes encoding these proteins span the segments of repeated DNA sequence flanking a region of long unique sequence. Thus, the first 62 amino acids of pp24 and pp38 are identical but the rest of the proteins are quite different. To understand the function of these genes, we plan to interrupt each gene by inserting a marker gene into the unique regions of each protein. Finally, we plan to interrupt both genes by inserting our marker gene in front of the region that is common to both genes. The marker gene that we plan to use encodes a modified Green Fluorescent Protein, a naturally-fluorescent protein originally isolated from a jellyfish, *Aequorea victoria*. This marker gene will allow us to follow the mutant MDVs through their infection of chickens to identify what abilities may be lost when the pp24- or pp38-encoding genes are disrupted. Through our study of these viruses, we hope to learn the function of these genes as they relate to MDV infection, pathogenicity and tumor-formation.

9702507 Role of a Pore-Forming Toxin in Pathogenesis of Actinomyces Pyogenes Infection
Billington, S.J.; Songer, J.G.; Jost, B.H. **Grant 97-35204-4750**

University of Arizona
Department of Veterinary Science and Microbiology **$192,000**
Tucson, AZ 85721-0000 **2 Years**

Actinomyces pyogenes causes a plethora of diseases in pigs, cattle and other animal species. Usually found as a secondary invader following a prior infection or trauma, *A. pyogenes* can also be a primary pathogen. It causes both acute and chronic purulent infections including liver abscess, mastitis, osteomyelitis, postpartum uterine infections, pneumonia and septic arthritis. The development of an effective vaccine against this organism requires a thorough understanding of the virulence factors of *A. pyogenes* and their role in immunity. *A. pyogenes* secretes a potent red blood cell-lysing toxin, pyolysin, which is similar to members of a family of toxins (the thiol-activated cytolysis), which are important in the disease process of other pathogenic bacteria. Hence, we hypothesize that pyolysin plays an important role in the virulence of *A. pyogenes*. Our studies will concentrate on inactivating the gene encoding pyolysin in *A. pyogenes*, thus allowing demonstration of a role for this toxin in the disease process. Site-specific and random mutations of single amino acids within pyolysin will allow characterization of residues important for activity of the toxin. As passive protection experiments in mice suggest that antibodies to pyolysin are protective, mutant pyolysin molecules which have reduced toxicity, but retain antigenicity, will be tested in animals as a vaccine against *A. pyogenes* infection.

9702736 Stress: Impact on Animal Well-Being
Moberg, G.P.; Mench, J. **Grant 97-35204-4857**

University of California, Davis
Department of Animal Science
Davis, CA 95616-8571
$5,000
1 Year

Partial support will be provided for an international conference on animal stress which will address five critical areas related to developing the basis for understanding the impact of stress on animal well-being: 1) what are meaningful measures of stress in animal, 2) what constitutes long-term stress in animal, 3) the cognitive and motivational aspects of animal stress, 4) the comparative, evolutionary and environmental aspects of stress, and 5) what can be done to reduce animal stress. The goal of the conference is to facilitate the exchange of research data and ideas between experts in basic stress research, human-biomedical research, animal welfare and applied animal research. The primary focus will be directed at how behavior related stressors, which are of special concern to animal agriculture, impact animal well-being. Unlike environmental stressors, behavior related stressors are still poorly defined and the correlation between such stressors and the biological well-being of animals in unclear. Questions concerning the role of cognition and motivation, what constitutes long-term stress in animals and potential strategies to alleviate stress must be addressed if we are to develop management practices for food animals that will insure the well-being of these animals.

9702371 Phenotypic Characterization of Equine Arteritis Virus with an Infectious cDNA Clone
Balasuriya, U.B.R.; MacLachlan, N.J. **Grant 97-35204-4736**

University of California, Davis
Department of Veterinary Pathology, Microbiology and Immunology
Davis, CA 95616
$176,689
3 Years

Equine arteritis virus (EAV) is distributed throughout the world and causes periodic outbreaks of equine viral arteritis (EVA). The disease is characterized by "flu-like" illness in horses, abortion in pregnant mares, and persistent infection in stallions. Persistently infected stallions are the critical reservoir of EAV, and during the course of persistent infection novel virus variants with different virulence characteristics are selected. Novel genetic variants that arise during persistent infection of stallions are spread to susceptible mares during breeding, and these novel variants then likely are spread via the respiratory route to cause some epizootics of viral arteritis. An infectious, full-length, complementary DNA copy (cDNA) of EAV viral genomic RNA recently was produced in the Netherlands. This cloned cDNA copy of the EAV genome can initiate a complete, productive infectious cycle in susceptible mammalian cells and is a powerful tool to study the EAV at the molecular level. Through collaborative arrangement with the University of Leiden we have acquired this infectious cDNA copy of EAV. We will introduce specific nucleotide changes using reverse genetics techniques (e.g. site-directed mutagenesis) into this cloned cDNA copy to study the genetic basis of antigenic variation, pathogenesis and virulence of EAV. Specifically, it is possible to test progeny virus for specific phenotypic manifestations of site-directed mutations and recombinations (chimeric viruses) which have been introduced into the cDNA clone. Similarly, development of an attenuated EAV strain using the infectious cDNA might ultimately also be used as a stable validated repositories of seed viruses for live virus vaccine production or for the construction of a genetically marked live virus vaccine.

9702613 A Quantitative Risk Analysis on the Movement of Animal Pathogens through the Import or Export of Infected Animals
Carpenter, T.E.; Gardner, I.A.; MacLachlan, N.J.; Johnson, W.O. **Grant 97-35204-4772**

University of California, Davis
Department of Medicine and Epidemiology
Davis, CA 95616
$190,000
3 Years

In the future, the sustainability of agriculture and animal industry within the United States will depend on the ease of moving animals and animal products both interstate and internationally. One barrier to unrestricted access of animals and animal products is the threat of infectious diseases that are foreign to the importing country or region. This project will address the issue of disease introduction through animal importation and exportation by generating general, quantitative risk assessment models. These models will then be applied to the movement of bluetongue virus (BTV), a vector-borne disease of livestock. We will assess the risk of introducing BTV to the United States through the importation of live animals, we will estimate the risk of spreading foreign isolates of BTV should they enter the United States in order to ease export restrictions in low risk regions. The final version of these models well be incorporated into a user-friendly environment enabling it to act as a generic risk assessment model for the import and export of other diseases, a need expressed by many different organizations.

9702635 Construction of a GroESL Mutant of Pasteurella as a Vaccine for Avian Cholera
Hirsh, D.C.; Wakenell, P.S.; Carpenter, T.E. **Grant 97-35204-4511**

University of California, Davis
Department of Pathology, Microbiology, and Immunology **$135,000**
Davis, CA 95616-0000 **2 Years**

The bacterium, *Pasteurella multocida* produces an economically important disease in poultry, avian cholera. Current vaccines used by the industry are not satisfactory (labor intensive, sometimes disease producing).

Turkeys, as do all avian species, have high body temperatures. Microorganisms placed at such temperatures exhibit a "heat shock response" by producing heat shock proteins that allow growth at increased temperature. Heat shock proteins and genes encoding them are highly conserved amongst the microbial kingdom. Microorganisms unable to produce a "heat shock" response are unable to grow at increased temperatures. *P. multocida* produces a classic "heat shock response". We hypothesize that a reduction in the heat shock response by *P. multocida* will result in a reduction of the microorganism's ability to grow in turkeys, and such strains would be unable to produce disease. The objective and approach for the proposed research is to construct a modified, live vaccine for avian cholera by constructing a mutant of *P. multocida* that is unable to produce a "heat shock" response by deleting the genes for the heat shock proteins. Such a vaccine would be easy to administer (drinking water) and safe.

The significance and novelty of our research goes beyond the study of an infectious disease of fowl. Producing a mutation in genes that are highly conserved resulting in a reduction in virulence sufficient to immunize without producing disease, introduces a method to produce a vaccine that could be used with any bacterial pathogen affecting any species of animal including humans.

9702608 Cohort Study of Shoeing and Risk of Injury and Lameness in Racehorses
Stover, S.M.; Gardner, I.A. **Grant 97-35204-4963**

University of California, Davis
Department of Anatomy, Physiology and Cell Biology **$200,00**
Davis, CA 95616-8732 **2 Years**

A study following 150 horses for 3 months at a racetrack will determine the types of shoes worn by Thoroughbred racehorses in California, and the incidences of an inability to race or train due to lameness, and/or musculoskeletal injury, will be identified. It is anticipated that results will be used to formulate recommendations for racehorse shoes that will decrease the incidence of lameness and musculoskeletal injury, and thus the economic and emotional hardships associated with lost training time, potential race earnings, medical and surgical treatment, and rehabilitation after injury. Potential rewards include a reduction in the risk of injury to horses and riders, enhancement of the financial feasibility of owning racehorses, and creation of a positive public perception of the horse racing industry.

9702275 Immunity to *Tritrichomonas foetus*
Corbeil, L.B. **Grant 97-35204-4771**

University of California, San Diego
Department of Pathology **$230,000**
San Diego, CA 92103-8416 **2 Years**

The long term goal of this research is to prevent reproductive failure in cattle caused by genital infections. Trichomoniasis, a sexually transmitted disease caused by *Tritrichomonas foetus*, is studied because the incidence is high and the economic loss is great. An immunological approach is taken to prevent bovine trichomoniasis because a partially protective killed cell vaccine has demonstrated that immunoprophylaxis is possible and we have identified a protective antigen (TF1.17) which is abundant on the surface of *T. foetus*. Preliminary studies showed that the protective immunoaffinity purified TF1.17 antigen cross reacted strongly with chemically purified *T. foetus* surface antigen (SA). Therefore, the aims are to determine whether SA is as effective as TF1.17 antigen in immunizing cattle and to study mechanisms of local immune responses in the female bovine reproductive tract. The subcellular changes in the genital mucosa during trichomoniasis and the ability of isotypic antibodies to TF1.17 antigen to prevent attachment and/or cytotoxicity of *T. foetus* for uterine epithelial cells will be studied. The proposal is multidisciplinary because it involves three morphologic pathologists, and theriogenologist, two microbiologist/immunologists, a statistician and a biochemist. It is mission oriented because it addresses a sustainable approach to preventing an economically significant disease (trichomoniasis) in cattle.

9702631 Genetic Basis for Antigenic Variation in *Babesia bovis*
Allred, D.R. **Grant 97-35204-4768**

University of Florida
Department of Pathobiology **$154,000**
Gainesville, FL 32611-0880 **2 Years**

Many pathogenic agents are capable of establishing chronic infections, even in hosts who are immune to disease. *Babesia bovis*, a protozoal parasite of cattle, is an example. From a single exposure *B. bovis* can establish an infection of several years duration. One mechanism used by infectious agents to evade the immune system is to rapidly alter certain components, a process called antigenic variation, rendering the host immune system unable to control the agent. The VESA1 antigen of *B. bovis*, a parasite component placed on the surface of the red blood cell in which the parasite lives, undergoes such antigenic variation. Although the native form of the antigen on the red blood cell surface is not cross-reactive among antigenic variants when assayed with variant-specific immune sera, the denatured form of the antigen is cross-reactive. We propose that the gene encoding the VESA1 a subunit is part of a multigene family, the various members of which share considerable sequence and structural homology. We propose to test this by characterizing several different expressed forms of the *ves1* gene that encodes the VESA1a polypeptide. The *ves1* gene will be isolated from multiple antigenically variant but otherwise genetically identical parasites that share a grandmother-mother-daughter relationship. The parasites varied *in vivo* and their native VESA1a polypeptides are not cross-reactive. These analyses will allow determination of how much the structure and sequence of the *ves1* gene may vary during chronic infection, and will help to identify any sequences or structures that may be conserved. This information will further our understanding of the mechanisms used by infectious agents to establish chronic infectious or to achieve breakthrough infections in vaccinated animals. This knowledge will benefit U.S. agriculture by assisting the development of strategies to circumvent this mechanism of immune evasion, potentially in a wide range of infectious agents. This could facilitate the development of vaccines or pharmacologic agents that target this immunologically-sensitive class of antigens.

9702241 Assembly of the Novel MSP-based Motile Apparatus of Nematode Sperm
Roberts, T.M. **Grant 97-35204-4860**

Florida State University
Department of Biological Science **$142,000**
Tallahassee, FL 32306-3050 **2 Years**

Parasitic roundworms, or nematodes, are important pathogens of all types of livestock. Resistance to drugs and limited effectiveness of vaccines have made it difficult to combat nematode infections and emphasized the need for a better understanding of the basic physiology of these organisms. For example, the reproductive capacity of nematodes plays a key role in the spread of infections but this process has not been studied extensively. The purpose of this proposal is to study the mechanism of sperm motility in the pig intestinal parasite, *Ascaris suum*. These unique sperm crawl like amoebas. Locomotion depends a novel system of filaments constructed from molecules of major sperm protein (MSP). We will purify MSP from sperm and analyze filament formation in the test tube under defined chemical conditions. To complement these studies of polymerization of purified protein, we will also examine how filament construction is controlled inside the cell. We have detected a chemical modification of a protein at the surface of sperm that coincides with filament assembly. We will use a combination of microscopic analyses and pharmacological assays to determine if this modified protein regulates the filament formation that enables the cell to crawl. These basic studies of sperm motility will enhance our knowledge of nematode reproduction and may provide the basis for designing ways to block fertilization and thereby disrupt transmission of the diseases cause by these organisms.

9702284 International Endophyte/Grass/Animal Toxicosis Symposium
Hill, N.S.; Thompson, F.N.; Stuedemann, J.A. **Grant 97-35204-4193**

University of Georgia
Department of Crop/Soil and Physiology/Pharmacology **$7,500**
Athens, GA 30602 **1 Year**

Tall fescue (*Festuca arundinancea Schreb.)* is grown on approximately 35 million acres of pasture in the United States. Nearly all tall fescue pastures are infected with a mutualistic endophytic fungus, *Neotyphodium coenophialum*, that produces ergot alkaloids. Animals grazing endophyte-infected tall fescue pastures ingest ergot alkaloids. Animals grazing endophyte-infected tall fescue pastures ingest ergot alkaloids and suffer from a disorder known as *fescue toxicosis*. Fescue toxicosis costs U.S. livestock producers $789 million annually due to reduced productivity. The endophyte provides tall fescue resistance to biotic and abiotic stresses, increasing its region of adaptation. Therefore, producers utilizing tall fescue pastures are trapped in a biological dilemma of utilizing endophyte-infected tall fescue pastures and suffering animal losses or use non-persistent but endophyte-free pastures. Other endophyte-mediated livestock toxicoses occur worldwide. Because of the complexity of the interactions between endophytes, their grass hosts, livestock, and the environment, unique strategies for resolving these toxicoses have been developed by scientists from around the world in different research disciplines. Plant breeding, immunology, and

endophyte manipulation appear to be possibilities for resolving the toxicoses. This project will help support an international symposium that will provide a forum for exchange of the current status of research efforts in ecology and taxonomy of endophytes, endophyte-derived livestock toxicoses, cellular and molecular techniques for plant and animal studies, commercial uses of endophytes, and current recommendations for alleviating toxicoses on the farm. The findings/recommendations will be debated and future research avenues explored. A bus tour of ongoing cooperative research between The University of Georgia and USDA-ARS will be conducted.

9702607 Virulence Factors of Enterotoxigenic *Escherichia coli*
Issacson, R.E. **Grant 97-35204-4510**

University of Illinois, Urbana
Department of Veterinary Pathobiology **$140,000**
Urbana, IL 61802 **2 Years**

Diarrheal disease caused by enterotoxigenic *Escherichia coli* (ETEC) is a common cause of mortality in young animals. The production of disease is dependent on the expression of adhesins (pilus-adhesins) that facilitate the colonization of small intestines. Current strategies to control or prevent ETEC induced diarrhea are based on the prevention of colonization through the used of pilus-adhesins as vaccines. Our data indicates that in nature the suppression of pilus-adhesin expression occurs and leads to the emergence of a new pilus-adhesin called F41. The emerging picture of pilus-adhesin expression is very complex and shown to be dependent on environmental conditions with production being maximally stimulated by the conditions in the animal. For example, K99 expression appears to maximal at 37C and in rapidly growing cells which is similar to what occurs during infection. We also have found that a mutation in or near a gene called *rnpA* (encodes ribonuclease P) suppresses K99 production. It is believed that an understanding of pilus-adhesin expression will provide valuable information on ETEC pathogenesis and potential new strategies to control disease. The specific aims are: 1) to study the role of *rnpA* or linked genes in K99 expression, 2) to study growth phase regulation of K99, and 3) to investigate the mechanism of thermal regulation of K99.

9702654 Prevention of Neutrophil Damage in Bovine Pulmonary Pasteurellosis
Ackermann, M.R. **Grant 97-35204-4769**

Iowa State University
Department of Veterinary Pathology **$155,000**
Ames, IA 50011-1250 **2 Years**

Spontaneous and experimental infection of cattle with the bacteria *Pasteurella haemolytica* induces excessive infiltration of white blood cells, specifically neutrophils, into the lung. The neutrophils release enzymes and other compounds that damage the lung parenchyma and impair the exchange of air. Existing clinical therapies rely on antibiotics to treat the bacteria but do not inhibit the neutrophils in bovine pulmonary pasteurellosis (BPP), the molecules that mediate neutrophil infiltration into lung have not been fully characterized. In this proposal we use a bovine model of BPP to identify adhesion molecules present on the surface of neutrophils that mediate neutrophil infiltration into the lung airways. We then use therapies that inhibit the adhesion molecules vital to this process and protect against BPP. One therapy will utilize a compound that inhibits adherence of carbohydrates-like molecules; a second therapy will use compounds that inhibit adherence of peptides. These new types of therapies for BPP that will augment antibiotics and other existing clinical treatments.

9702642 Gordon Conference: Biology of Spirochetes
Zuerner, R.L. **Grant 97-35204-4509**

USDA Agricultural Research Service
Zoonotic Diseases Research Unit **$7,500**
Ames, IA 50010-0070 **1 Year**

Funds are sought for support of the third Gordon Conference on the Biology of Spirochetes. This is a unique international conference which brings together investigators working in all areas of spirochete biology. Recent technological advances in molecular biology and immunology have been applied with great success to studying these bacteria. There is a critical need for researchers to exchange new information. The two previous Gordon Conferences on the biology of spirochetes have led to greater collaborative efforts and reduced duplicative research.

Spirochetes are bacteria which often cause chronic infections. These infections are usually difficult to diagnose or treat. Leptospirosis, swine dysentery, intestinal spirochetosis of pigs, and a colitis of poultry are important to the livestock and poultry industries and cause significant economic losses. Spirochetes are also the likely causes of epizootic bovine abortion and papillomatous digital dermatitis (hairy footwart).

The conference will focus on research at the forefront of spirochete research. Several diverse topics will be covered including virulence factors, interactions with host cells, host responses to infection, ecology, and regulation of gene expression. Because these bacteria share many similarities, research on one group of spirochetes often leads to new discoveries with other spirochetes.

9702612 The Biological Significance of the Hingeless Allelic Variant of Porcine IgA
Butler, J.E. **Grant 97-35204-4858**

University of Iowa Medical School
Department of Microbiology & Interdisciplinary Immunology Program
Iowa City, IA 52242-1109
$190,000
3 Years

Pathogens which compromise the health of farm animals, such as swine, primarily enter via the digestive or respiratory tracts. Both systems depend on the mucosal immune system and IgA antibodies for protection. IgA is produced in larger amounts than all other classes of antibodies, constitutes 80-90% of antibodies associated with the intestinal and respiratory mucosa and is the major class of antibody transmitted in the milk to suckling piglets.

The genetics of porcine IgA are unique, since one of the two genetic variants (IgA^b) lacks two-thirds of the normal hinge. The hinge of an antibody is believed necessary for proper molecular flexibility when binding pathogens and for being transported across mucosal epithelia. However, a short hinge may be advantageous in rendering IgA less susceptible to bacterial proteases.

This is a proposal to determine if IgA^b is a negative survival trait or actually advantageous to pigs under certain circumstances. Specifically, we shall examine differences in IgA protease susceptibility, transport across epithelial membranes, affinity for IgA-binding proteins on certain bacterial pathogens and expression in developing piglets.

The study is relevant to the long-term goals of agriculture through the functional characterization of a genetic trait in an agriculturally important species that may determine resistance/susceptibility to mucosal pathogens and perhaps, help characterize potential virulence factors in swine pathogens.

9702286 Type 1 Fimbriae of *Salmonella* serovars; Colonization Antigens and Adhesins
Clegg, S. **Grant 97-35204-4616**

University of Iowa
Department of Microbiology
Iowa City, IA 52242
$200,000
2 Years

Salmonella typhimurium is a major cause of bacterial food-poisoning and the reservoir of human infections is agriculturally important animals. Specifically, infected poultry and swine represent a significant source of bacteria causing human infections. *Salmonella* serovars that are isolated from poultry commonly express a specific type of surface molecule that has been implicated in facilitating colonization of the gastrointestinal tract. Our group has been able to clone and characterize the genes encoding this adherence factor, and we have constructed defined mutations in this gene cluster. Using these recombinant DNA molecules it has been possible to generate defined *Salmonella* mutants that are altered in the expression of the adherence factor. We will use these mutants to investigate the role of the adherence factor in mediating bacterial attachment, *in vivo,* to the intestinal mucosa of poultry. Also, the ability of isogenic strains of *S. typhimurium,* that differ only in the presence of surface adherence factors, to be transmitted between individual animals will be determined. Since transmission of *Salmonella* serovars among flocks of poultry is important in the dissemination of the bacteria and therefore subsequent human exposure, mechanisms that can block or inhibit this process will have broad public health significance. The objectives of our research are to examine the mechanisms of long-term carriage of *S. typhimurium* by infected poultry and to determine the role of adherence factors in facilitating interanimal transmission. The reduction of *Salmonella* carriage by poultry will be of tremendous economic benefit to US agriculture.

9702508 BHV-1 and BHV-5 Neuropathogenesis Studies
Chowdhury, S.I.; Mosier, D.; Kennedy, G.A. **Grant 97-35204-4700**

Kansas State University
Department of Diagnostic Medicine and Pathology
Manhattan, KS 66506-0000
$180,000
3 Years

Bovine herpesvirus 1 (BHV-1) also known as infectious rhinotracheitis virus (IBR) and BHV-5 also known as bovine encephalitic herpesvirus (BEHV) are significant viral pathogens of cattle. Both of the viruses are neurotropic and establish latency in sensory neurons. However, they differ in their ability to cause neurological disease in calves. Little is known about the mechanism of differential neuropathogenesis of these viruses. Genetically, these two viruses are closely related (85% DNA homology) yet cause distinct disease in cattle. A detailed understanding of the basic molecular mechanism of disease is important in designing prophylactic vaccines against the disease. We have established a rabbit seizure model that distinguishes between BHV-1 and BHV-5 acute neurological infections.

The goals of the proposed research are to generate BHV-1 and BHV-5 glycoprotein (g) gE recombinants and to compare and analyze the pathogenic properties of these mutants in the rabbit model. Additionally, gC and gD recombinant viruses developed under the auspices of our USDA grant 95-37204-2309 will be similarly evaluated. The proposed study will help: 1) to identify viral genes that are involved in determining the differential pathogenic properties of BHV-1 and 5, 2) to understand the role of these glycoproteins in BHV-5 mediated neuropathogenesis, and 3) to design prophylactic vaccines against the disease.

9702626 Regulation and Function of Interleukin 6 in the Porcine Anterior Pituitary
Minton, J.E.; Grieger, D.M. **Grant 97-35204-4962**

Kansas State University
Department of Animal Sciences and Industry **$154,000**
Manhattan, KS 66506-0201 **2 Years**

The goal of this proposal is to enhance understanding of interactions among hormonal and immune mechanisms which contribute to the response of a host animal to disease challenge. The research focuses specifically on two areas. One set of studies is aimed at investigation of cellular mechanisms within the anterior pituitary gland that may alter hormone secretion and impact health and growth in infected pigs. The second series of studies are designed to investigate inter-relationships between immune responses (systemic pro-inflammatory cytokine production) and hormonal responses (stress hormones and hormones associated with normal growth promotion) in experimental models of *Salmonella* infection in young pigs. These studies overlap fundamentally because the pituitary gland is required for survival to *Salmonella* infection. Salmonellosis is a disease of major economic importance to the swine industry in the United States costing the industry and estimated $100 million annually. In addition, food-borne sources of *Salmonella*, including meat products, are of importance in human disease. Thus, the proposed studies have relevance to animal agriculture within the U.S. Finally, as this research is aimed at developing a greater understanding of cellular and molecular responses of livestock in disease models, additional knowledge in the area may be useful in identifying traits of importance to disease resistance. Such knowledge may be of use in traditional selection applications or in conjunction with newer technologies to aid in selection of traits which enhance disease resistance of livestock.

9702260 Regulation of Bovine Gamma/Delta T Cells
Black, S.J.; Baldwin, C.L. **Grant 97-35204-4909**

University of Massachusetts
Department of Veterinary and Animal Sciences **$154,550**
Amherst, MA 01003-6410 **2 Years**

T lymphocyte-mediated immune responses are important for fighting infectious diseases. Understanding of how T lymphocytes interact with agents that cause disease is required for the development of vaccination strategies. There are 2 major categories of T lymphocytes: the alpha/beta T cells and the gamma delta T cells. Alpha/beta T cells were discovered first and have been extensively characterized. They respond to foreign bacterial, virus and parasite components known as antigens, presented by ones own cells. Effective strategies are being developed to prime or suppress alpha/beta T cell responses based on this knowledge. Antigen recognition by the gamma/delta T cells different from that of the alpha/beta T cells: the molecules that gamma/delta T cells respond to are not necessarily presented by ones own cells and they may be very different in their chemical nature from those recognized by alpha/beta T cells. Characterization of gamma/delta T cell stimulatory antigens and definition of how these molecules interact with the gamma/delta T cell antigen receptor is crucial to gaining insight into how to manipulate the cells by vaccination or immunotherapeutic regimes. Our data indicate that the principal stimulatory entity for bovine blood gamma/delta T cells is a constitutively expressed molecule of blood monocytes. The objective of the study is to purify the monocyte membrane component that induces responses by bovine gamma/delta T cells. This will expedite our long term project goal which is to define the antigenic stimuli, function and regulation of gamma/delta T cells in cattle.

9702414 Infectious Bovine Respiratory Syncytial Virus from Cloned cDNA: Potential for Vaccine Development and Basic Studies
Samal, S.K. **Grant 97-35204-4916**

University of Maryland
VA-MD Regional College of Veterinary Medicine **$180,000**
College Park, MD 20742-3711 **3 Years**

Bovine respiratory syncytial virus (BRSV) is one of the most important causes of respiratory tract disease in beef and dairy cattle. The disease causes serious economic losses to the cattle industry and is considered the single most important disease problem of the cattle industry. Currently available vaccines for BRSV are not satisfactory. Therefore, there is a great need to develop a satisfactory vaccine against BRSV infection. Our long-range goal is to develop safe, effective and inexpensive live attenuated vaccines for BRSV infection using genetic engineering techniques. These vaccine viruses will be generated in the laboratory by directly introducing irreversible changes into the genetic material (nucleic acid) and of the virus. Unlike currently available BRSV vaccines, our vaccine viruses are less likely to revert back to virulence. As a first step toward this goal we propose to construct a full-length copy of the genetic material of BRSV in our laboratory so that it can be manipulated in the future. The full-length copy of the genetic material of BRSV in our laboratory so that it can be manipulated in the future. The full-length copy of the BRSV genetic material will be used to produce infectious BRSV in cell culture using recently available reverse genetic techniques. Later, site-specific irreversible changes will be introduced into the full-length copy of BRSV genetic

material and attenuated vaccine viruses will be produced in our laboratory. The results of this project will have important applications for the development of a new generation of attenuated BRSV vaccines by direct genetic manipulation.

9702492 Molecular Basis of Pathogenesis and Virulence in Infectious Bursal Disease Virus
Vakharia, V.N. **Grant 97-35204-4910**

University of Maryland
Center for Agricultural Biotechnology **$200,660**
College Park, MD 20742-4450 **3 Years**

Infectious bursal disease virus (IBDV) is a pathogen of major economic importance to the nation's twenty billion dollar poultry industry. The virus attacks the major immunological organ of young chickens which results in severe immunosuppression and death. The overall goal of our investigation is to identify the viral gene(s) involved in causing this disease. We have developed a system in our laboratory with which we can prepare a "tailor-made" virus (IBDV) using the recombinant DNA techniques. Using this method, we will prepare chimeric viruses of a vaccine and virulent IBDV strain by swapping their gene(s). We will then evaluate the properties of chimeric viruses in chickens and identify the gene(s) involved in causing pathogenesis and virulence. The results of our study will provide the poultry industry with critical reagents for future vaccine development and minimize flock losses should highly virulent strains of IBDV emerge in the United States.

9702418 The Immunobiology of Chicken Interleukin-2/15
Sundick, R.S. **Grant 97-35204-4914**

Wayne State University
Department of Immunology and Microbiology **$152,406**
Detroit, MI 48201 **3 Years**

The goal of this research is to characterize the immunobiological properties of a gene that we have recently cloned. The gene was isolated from a chicken spleen cDNA expression library, using an assay detecting factors inducing the proliferation of activated chicken T cells. Sequencing of the gene revealed that it has significant amino acid homology to two mammalian lymphokines, IL-2 and IL-15. These mammalian lymphokines have similar effects on cells of the immune system; inducing the proliferation and/or differentiation of activated T, B, and NK cells. Mammalian IL-15 also has properties not shared by mammalian IL-2; e.g. it stimulates muscle cells to produce myosin. To determine the properties of our cloned chicken gene (IL-2/15), we will produce quantities of the recombinant protein and test its effects on T, B, and NK cells. The protein will also be used to produce antibody, enabling the purification of native IL-2/15 protein from activated chicken spleen cells. The native IL-2/15 protein will be characterized biochemically and compared with recombinant IL-2/15 for its *in vitro* effects on lymphoid cells. Studies will also be performed to determine the types of cells producing this protein and the kinetics of gene expression following mitogen stimulation. This research should have a significant impact on our understanding of the avian immune system and our ability to manipulate it, both *in vitro* and *in vivo*. This gene (or gene product) has the potential to improve the efficacy of chicken vaccines, particularly when administered *in ovo* or at hatching.

9702383 Regulation of Marek's Disease Virus (MDV) Transformation and Replication
Lee, L.F.; Kung, H.J.; Witter, R.L. **Grant 97-35204-4937**

USDA, Agricultural Research Service
Avian Disease and Oncology Lab **$161,000**
East Lansing, MI 48823-5338 **2 Years**

Marek's disease (MD) is a T-cell cancer of chickens caused by a herpesvirus. Despite expensive vaccination programs, it still remains an economically important disease of chickens due to the continued emergence of increasingly more deadly strains of Marek's disease virus (MDV) in the field. The long-term goal of the project is to define the multifunctional aspects of an oncogene (gene that causes tumor), MEQ, in tumor induction and maintenance of the latent (hidden) state of the virus. In addition, we wish to determine the involvement of MEQ gene in regulating MDV replication (reproduction). The significance of the proposed study lies in its contribution to basic knowledge and the application of this knowledge to disease control. Findings are expected to help discover the fundamental concepts governing studies on basic understanding of MDV at the gene structure and regulation of gene expression levels, both of which are lacking in Marek's disease research at the present time. By determination of the biochemical nature of the MEQ product, we will uncover its role in tumor development, and demonstrate the feasibility to genetically manipulate tumor cells and gene replacement therapy, such as the development of chickens resistant to MD. This work has the potential for yielding enormous economic benefits for the nation's largest agricultural product in the poultry industry.

9702235 Marek's Disease Virus: Identification of Oncogenic Genes
Silva, R.F.; Cheng, H.H.; Witter, R.L. **Grant 97-35204-4699**

USDA, Agricultural Research Service
Avian Disease and Oncology Laboratory
East Lansing, MI 48823-5338

$160,000
2 Years

The poultry industry spends over $15 billion a year to prevent and control infectious diseases. One of the most economically devastating infectious diseases of poultry is Marek's disease (MD), caused by Marek's disease virus (MDV). Although chickens with MD typically develop lymphomas and subsequently die, there is often great variability in both the incidence and severity of disease at different localities. It is now known that some of the variability observed in severe MD outbreaks is due to the appearance of novel, highly virulent forms of MDV. Current vaccines appear to be ineffective against these new variants.

We propose to identify the MDV genes (hereditary units in the virus that are responsible for the different characteristics of MDV that are involved in lymphoma production by mixing vaccine strains of MDV together with short pieces of DNA isolated from virulent viruses. By noting which DNA fragments restore virulence to the vaccine virus, we can pinpoint which genes are responsible for virulence. Once we have identified which MDV genes are responsible for MD in chickens, we will be able to mutate (induce a change in the DNA sequence of a gene) these genes in the new virulent MDV isolates, thereby generating non-virulent viruses that can be used as vaccines.

9703082 Characterization of the bovine P2XA$_7$ Receptor in Cattle
Smith, R.A. **Grant 97-35204-4913**

University of Missouri, Columbia
Department of Veterinary Pathobiology
Columbia, MO 65211-0000

Postdoctoral Award
$90,000
2 Years

A bovine tuberculosis eradication program in the United States has substantially reduced the number of tuberculin reactive cattle since its implementation in 1917. However, the complete eradication of *Mycobacterium bovis* has not been possible because similar programs do not exist or are not as stringent in other American countries. *M. bovis* transmission from infected foreign cattle to U.S. herds exists and has been a problem for herds along the U.S.-Mexico border. In addition, the nature of *M. bovis* allows the transmission from humans and wild and exotic domestic animals to cattle. A general understanding of how mycobacterium, in particular *M. bovis*, is killed by the immune system is essential for determining the most effective routes for eliciting and immune response that will efficiently clear mycobacterium infection and can potentially be used in vaccine protocols. *M. bovis* resides inside of monocyte/macrophage cells of the animals they infect. ATP-induced cell death of monocytes is associated with the intracellular killing of the Calmette-Guerin strain of *M. bovine.* Additionally, P2X$_7$, an ATP-regulated receptor is involved in cell death of murine macrophages. In this study, the bovine homolog of the P2X$_7$ receptor will be cloned and both the bovine and murine receptors will be characterized. This information will be used to evaluate the role of this receptor in mycobacterial clearance from infected cells and determine further potential value in vaccine strategies.

9702650 Genetic Analysis of Avian *E. coli* Virulence
Curtiss, R.; Dozois, C.; Stathopoulos, C. **Grant 97-35204-4512**

Washington University
Department of Biology
St. Louis, MO 63130-4899

$270,000
3 Years

Avian pathogenic *Escherichia coli* is the causative agent of airsacculitis, septicemia, pericarditis and perihepatitis in poultry, and the use of production intensive confinement housing has resulted in the emergence of this organism as one of the predominant bacterial diseases affecting the poultry industry. Specific strains of *E. coli* are associated with a number of human and animal diseases, and those of serotype 01, 02 and 078 predominate in avian colibacillosis. Recent studies have shown that unique DNA regions on the genome of pathogenic *E. coli* strains are associated with
virulence in a particular niche. Our long-term objectives is to establish the genetic basis for the ability of avian pathogenic *E. coli* to colonize the respiratory tract and cause systemic disease. Our specific objectives for the grant period are to: 1) continue to construct mutants with replacement of DNA that is unique to an avian pathogenic *E. coli* strain and determine whether loss of these unique regions reduces virulence and, 2) identify by complementation cloning, transposon mutagenesis and gene cloning the individual genes within unique DNA regions that contribute to virulence. The research will contribute to the basic knowledge towards the mechanisms by which *E. coli* causes disease in poultry. The research will make use of genetic, biochemical and animal science techniques.

9702461 Molecular Characterization of *Neospora* Antigens
Sibley, L.D. **Grant 97-35204-4770**

Washington University School of Medicine
Department of Molecular Microbiology
St. Louis, MO 93110-1093
$240,000
3 Years

Neospora caninum was first identified as a parasitic disease of dogs, but it is now recognized as a cause of abortion and newborn sickness and death in dairy cattle. The absence of characterized proteins that are unique to *Neospora* have hampered the development of reliable assays for the identification and diagnosis of neosporosis. Consequently, it has not been possible to initiate the epidemiological studies that are crucial for the formulation and implementation of preventative practices, and this disease therefore continues to be a problem for the dairy industry. The proposed research will address these deficiencies by identifying and characterizing at the molecular level two surface proteins of *Neospora*, designated p27 and p35, that react strongly with the immune system of infected animals. The genes that encode the p27 and p35 proteins will be identified and used to produce large quantities of these proteins in the laboratory. We will examine different strains of *Neospora* that have been isolated from dogs and cattle to determine if the p27 and p35 proteins are conserved. We will also test our diagnostic reagents by examining blood and tissues from animals infected with Neospora or other closely-related parasites. These studies will establish the feasibility of using these proteins for future development of diagnostic tests and as possible vaccines to prevent neosporosis.

9702356 Characterization of the *Toxoplasma gondii* Sporozoite-specific Surface Proteins p30 and p67
White, M.W. **Grant 97-35204-4859**

Montana State University
Department of Veterinary Molecular Biology
Bozeman, MT 59717-3610
$154,000
2 Years

The protozoan, *Toxoplasma gondii*, is a zoonotic parasite causing serious disease in man and animals, particularly of the unborn or immunologically deficient. Contained within the environmentally resistant oocyst, the *T. gondii* sporozoite stage is pivotal to the transmission of this disease. By way of oocyst contamination of water or soil, people and animals acquire this microorganism with the most common apparent livestock infections occurring in sheep and newborn pigs. Many more oocyst infections of livestock are inapparent which poses a second risk of infection to populations when contaminated food products containing the tissue cyst stage are consumed. Thus, understanding sporozoite biology is key to ultimately preventing the transmission of Toxoplasma to people and food animals. *T. gondii* sporozoites have evolved stage-specific mechanisms to survive the animal gut environment and to efficiently invade the intestinal mucosa. Consistent with this hypothesis, we have discovered a novel sporozoite invasion mechanism that involves stage-specific proteins. This project focuses on the earliest step in the sporozoite invasion of host cells and the potential role of the sporozoite-specific surface proteins, p30 and p67 in these mechanisms. Using antibodies directed against these molecules, we will employ adhesion and neutralizing assays to evaluate the role of p30 and pS7 in host cell attachment and penetration. We will isolate RNA at various times during oocyst sporulation and prepare a mixed sporozoite expression cDNA library. This library will then be screened in an attempt to clone the cDNA fragments encoding p30 and p67 for use in future genetic experiments.

9702239 Regulation of Mucosal Barrier Function in Infectious Enteritis
Argenzio, R.A. **Grant 97-35204-4494**

North Carolina State University
Department of Anatomy, Physiological Sciences & Radiology
Raleigh, NC 27606
$220,000
2 Years

The long term goal of our laboratory is to identify the cellular and molecular mechanisms of severe diarrhea and to determine specific therapies which facilitate intestinal absorption and mucosal repair in infectious enteritis. Neonatal diarrhea accounts for the major proportion of economic losses suffered by the pork and dairy industries. Diarrheal dehydration is the leading cause of infant mortality worldwide. Combined pharmacologic and oral rehydration therapy that targets both mucosal absorption and repair from excessive inflammatory responses can be-a practical and economical means of reducing morbidity and mortality in these acute diarrheal diseases. We propose that prostaglandins (PGs) and the amino acid arginine are essential to maintenance and repair of the intestinal mucosal barrier by augmenting epithelial resistance and stimulating epithelial restitution, a process by which viable epithelial cells migrate over the injured mucosa. Current studies suggest that the protective prostaglandins are modulated by nitric oxide (NO), a product of arginine metabolism. We propose that activated phagocytes initiate mucosal damage via cytokine and oxidant production, however these products also induce NO and PG synthesis, which then protect and repair the mucosal barrier. We will study these mechanisms using both acutely injured and cryptosporidial-infected piglet tissue in Ussing chambers in vitro, which allow measurements of epithelial injury and cytokine and PG production rates by the tissue. In addition, we will study direct effects of PG and NO on epithelial migration rates in cultured epithelial monolayers. These studies should provide the rationale for optimal pharmacologic and nutritional strategies in infectious enteritis.

9702288 Cis-acting Elements in the Replication of the Bovine Viral Diarrhea Virus Genome
Donis, R.O. **Grant 97-35204-5068**

University of Nebraska
Department of Veterinary and Biomedical Sciences **$180,000**
Lincoln, NE 68588-0430 **2 Years**

Bovine viral diarrhea virus (BVDV) is ubiquitous in the U.S. and threatens the profitability of cattle farming. Advances in genetic engineering techniques provide the means to define new molecular targets to develop safer and more effective vaccines. In particular, the 5' region of the BVDV genome contains two potential targets: 1. A region of the BVDV genome located near the extreme 5' of the genome, known as the internal ribosome entry site (IRES), which is essential for viral replication. 2. A promoter for positive strand RNA synthesis located at the 5' end of the genome. In order to map and characterize these functional domains of the BVDV genome, we propose: 1) Mapping and functional analysis of the IRES element. A panel of BVDV 5' region mutations will be introduced in a reporter plasmid vector to be analyzed in a cell-free translation system and *in vivo* by transient expression in cultured bovine cells, 2) Identification, mapping and functional analysis of transcription promoter elements directing plus-strand RNA synthesis. Mutational analysis of the 5' region will be carried out by assessing the replication of a subgenomic BVDV reporter replicon as well as complete BVDV genomes bearing the relevant changes. The proposed research will provide a detailed physical and functional description of genomic segments driving translation and replication. Future *in vitro* and animal inoculation studies will reveal if alteration of the newly discovered functional elements in the 5' region of the BVDV genome give rise to virus strains suitable for vaccination purposes.

9702394 Functional Analysis of Bovine Herpesvirus Latency Related Gene Products
Jones, C. **Grant 97-35204-4911**

University of Nebraska
Department of Veterinary and Biomedical Sciences **$248,452**
Lincoln, NE 68583-0905 **3 Years**

Bovine Herpesvirus 1 (BHV-1) is a significant viral pathogen of cattle which can cause upper respiratory disease, abortions, "shipping fever", or occasionally encephalitis. BHV-1 establishes a latent infection in the peripheral nervous system, primarily sensory neurons located in trigeminal ganglia, of an infected host. Virus can persist in a latent state for the lifetime of the infected host or can periodically reactivate. The ability of the virus to establish a latent infection and reactivate from latency is the main reason why BHV-1 is maintained in the field. During a latent infection, viral gene expression is limited to a single latency related (LR) gene.

Although it is believed that LR gene products regulate latency, a functional analysis of LR gene products has not been performed. Previous studies have determined that the LR gene encodes a protein which is expressed in neurons. The ability of LR gene products to prevent proliferation of cells and to bind cell cycle regulatory proteins is believed to promote neuronal survival when BHV-1 infects neurons. Since the LR rNA is alternatively spliced in neurons during infection, these alternatively spliced rNAs may encode protein isoforms which have novel functions. The goal of this grant is to elucidate the function of LR gene products and correlate these findings to latency. Understanding the function of this gene may lead to innovative anti-viral therapies which can prevent latency or eliminate reactivation from latency.

9702646 Can a DNA Vaccine Induce Cutaneous Immunity in Fish?
Clark, T.G.; Dickerson, H.W. **Grant 97-35204-4481**

Cornell University
Department of Microbiology & Immunology **$190,000**
Ithaca, NY 14853 **3 Years**

DNA vaccines have recently emerged as an important new tools in the fight against infectious disease. Such vaccines are composed of “naked” DNA which can be expressed as foreign protein following their introduction into recipient animals. In mammals, resulting immune responses have been shown to provide strong protection against a variety of microbial pathogens. Recent studies have suggested that DNA vaccines may be useful in aquaculture as well. To test this idea, attempts will be made to develop a DNA vaccine against *Ichthyophthirius multifiliis*, a protozoan parasite of freshwater fish. As the etiologic agent of “white spot”, *Ichthyophthirius* has substantial impact on commercial aquaculture in this country and abroad. In addition, it provides a unique model for the study of host-parasite interactions leading to immunity in fish. We have identified antigens on the parasite surface which can elicit protective resistance against *I. multifiliis* and have cloned the genes encoding these antigens. These genes will be modified for expression in teleosts and then introduced into channel catfish as recombinant DNA vaccines. The immune response to parasite antigens will be determined by measuring the levels of serum of serum and mucus antibodies in vaccinated fish, and the extent of protection afforded by such vaccines tested by direct parasite challenge. Aquaculture represents one of the fastest growing areas of agribusiness in the United States. Because fish raised under intensive farming

conditions are highly susceptible to infectious disease, the use of DNA vaccines may prove extremely useful in boosting productivity within this arena.

9702752 Vaccines for Poultry Using Antigens Produced in Transgenic Plants
Mason, H.S.; Suarez, D.L. **Grant 97-35204-5066**

Boyce Thompson Institute for Plant Research **$100,000**
Ithaca, NY 14853-1801 **2 Years**

Infectious diseases cause substantial losses to the poultry industry worldwide. In future outbreaks of disease in the U.S., vaccination may be considered for primary disease control. Because of the economics of the poultry industry, the vaccine must be inexpensive, easy to administer, and efficacious. The need for a feed-based orally administered vaccine is thus apparent. We propose to develop the use of transgenic plants for expression and delivery of recombinant subunit vaccines for poultry. We have already published several papers on the use of plants for production of vaccine antigens, and showed that when mice eat vaccine-containing transgenic potato tubers, they develop
antibodies against the recombinant tuber antigens. We will use avian influenza as a model for viral diseases in poultry, and hemagglutinin (HA), the major antigenic protein of the viral envelope, as a
recombinant antigen. We will first produce several different forms of HA in cultured tobacco cells, and determine which forms accumulate to the highest levels while maintaining the capacity to react with antibodies against authentic viral HA. We will then select the most promising HA forms for use in the creation of transgenic potato plants as a model whole plant system. We will verify the accumulation and antigenic structure of HA in the potato tubers, which will be used in later studies for feeding chickens in order to determine the vaccine efficacy. We anticipate that commercial development of plant-based vaccines for poultry will utilize grain crops, which are better suited for feeds.

9702753 Tissue Tropism and *in vivo* Persistence of Avian Infectious Bronchitis Virus
Naqi, S.A. **Grant 97-35204-5070**

Cornell University
Department of Microbiology and Immunology **$100,000**
Ithaca, NY 14853-6401 **2 Years**

Infectious bronchitis virus (IBV) causes a highly contagious, and economically significant infection of chickens in the United States and other poultry producing countries. Persistent IBV infection is a critically important problem in egg-type chicken flocks. Persistently infected chickens rarely attain their egg production potential, produce eggs of poor quality and serve as a source of infection for other birds. Equally important, IBV could undergo genetic change during persistent infection resulting in the emergence of new IBV strains with increased disease-producing potential and with ability to produce disease in IBV vaccinated chickens. Such "variant" strains of IBV have been a continuous threat to both commercial egg-type and meat-type chicken flocks. Presently, control of persistent IBV infection is difficult because factors which contribute to IBV persistence are poorly understood. Little is known about the length of time individual birds may shed IBV after infection, and whether individual chickens could maintain persistent infection in the absence of periodic reinfection. It is also unclear what tissues and cells harbor the virus during persistent infection. To answer these questions, we propose studying IBV shedding and persistence in individually housed chickens applying state of the art techniques. We also plan to determine whether IBV could undergo genetic change during long-term infection of chicken tissues. The ultimate goal is to use this knowledge to minimize persistent IBV infection in the field so that the U.S. poultry industry can maintain its economic competitiveness and provide inexpensive and safe food to consumers.

9702615 Pathobiochemistry of *Tritrichomonas foetus:* Bovine Trichomoniasis
Singh, B.N.; Gilbert, R.O.; Costello, C.E.; Burgess, D.E.; Levery, S.B. **Grant 95-37204-2234**

SUNY Health Science Center
Department of Biochemistry and Molecular Biology **$165,000**
Syracuse, NY 13210 **2 Years**

Bovine trichomoniasis is a sexually transmitted disease of cattle caused by the protozoan parasite *Tritrichomonas foetus.* The parasites are transmitted from the bull to the cow during coitus. This organism is an important pathogen in cattle resulting in fetal abortion and sterility of the cow. The disease is highly prevalent in the United States, especially where natural breeding is practiced. Bovine trichomoniasis causes considerable economic loss (est.> $1.4 billion annually) in the United States and as well as in other parts of the world. There is no effective diagnostic test or vaccine available for treating this disease. The main objective of this project is to study the *T. foetus's* cell surface glycoconjugates and their involvement in attachment of parasites to bovine vaginal epithelial cells (BVECs). We will also examine the hormonal effects on adhesion of parasites to BVECs. This research should help in defining the pathobiology of the trichomonad parasite. The trichomonad parasite possess novel types of carbohydrate containing molecule, the lipophosphoglycan (LPG) anchored on the cell surface via a lipid moiety. The experimental data suggest that this LPG molecule is involved in adhesion of parasites to BVECs. Also, the cattle infected with *T. foetus* shows

strong immune response to LPG molecule. Several chemical and enzymatic techniques will be applied to define the chemical nature/functions of these macromolecules. Knowledge acquired from these unique components that appear to be parasite specific could be exploited as targets for chemotherapy. Furthermore, studies will contribute to understanding the biological mechanisms of parasite pathogenesis and lead to development of diagnostic test and/or vaccines for bovine trichomoniasis.

9702638 Regulator of *Salmonella* Invasiveness
Maurer, R.A. **Grant 97-35204-4482**

Case Western Reserve University
Department of Molecular Biology and Microbiology **$150,000**
Cleveland, OH 44106-4960 **2 Years**

Salmonella species cause a spectrum of widespread infections ranging from gastroenteritis to typhoid fever. Both humans and feedstock animals are susceptible to infection, and the major source of human infection is by ingestion of contaminated food. Therefore, controlling Salmonella infections is important to protecting the health of feedstock animals, enhancing food safety, and ultimately protecting human health. The initial step of *Salmonella* infection, in which the bacterium attacks cells lining the intestine, requires the expression of a set of *Salmonella* virulence genes. This expression, in turn, is sensitive to a complex interaction of environmental and genetic factors (of the bacterium) and therefore represents a vulnerable point at which, at least potentially, the progression of *Salmonella* infections could be disrupted by well-designed interventions. In this project, a previously unknown *Salmonella* gene that participates in the regulation of virulence gene expression will be characterized, and its place in the overall regulatory scheme relative to other genetic and environmental factors will be investigated.

9702214 Lactogenic Immunity in Cows Vaccinated with Recombinant Rotavirus-like Particles
Saif, L.J.; Conner, M.E. **Grant 97-35204-4682**

The Ohio State University
Wooster Food Animal Health Research Program **$200,000**
Wooster, OH 44691-4096 **3 Years**

Rotaviruses are a major cause of diarrhea in humans and animals, including calves. In calves, diarrhea causes economic losses of $500 million/year, excluding treatment costs and production losses due to growth retardation. Because commercial rotavirus vaccines lack efficacy, it is timely to develop new vaccination strategies. Our goal is to develop multicomponent subunit vaccines for vaccination of cows to passively control rotavirus diarrhea in calves. Genetic engineering will be used to create novel rotavirus-like particle (VLP) vaccines that contain rotavirus core and surface proteins and are noninfectious, but induce immune responses similar to native virus. Furthermore, the rotavirus core-like particles will serve as a universal carrier for assembly of the surface proteins from the predominant rotaviruses (groups A,B,C) circulating in the field, thus creating hybrid rotavirus VLP vaccines capable of eliciting broader and more protective immunity. Updating and modifying rotavirus vaccines by adding new rotavirus surface proteins reflective of newly emergent field strains represents a novel vaccine concept. The VLP vaccines will be evaluated in cows by analyzing their antibody responses in blood and milk. The degree of passive protection provided by the mother will be assessed in rotavirus-challenged calves fed colostrum from vaccinated or control cows. Our approach should result in a new generation of rotavirus vaccines that are noninfectious but highly efficacious. Other advantages of VLP vaccines include the potential future production of vaccines for non-cultivatable rotaviruses and the possibility to modify rotavirus vaccines by incorporation of surface proteins from newly emergent field strains.

9702657 Fimbriae-mediated Adhesion of Enterotoxigenic *Escherichia coli*
Schifferli, D.M. **Grant 95-37204-1986**

University of Pennsylvania School of Veterinary Medicine
Department of Pathobiology **$165,000**
Philadelphia, PA 19104-6049 **2 Years**

Pathogenic microorganisms successfully initiate the infectious process by binding to animal surface molecules. Understanding these interactions at the molecular level will support the design of new and better preventive approaches against infectious agents. The best documented adhesive components of bacteria are hair-like surface appendages called fimbriae or pili. The long-term goal of this project is to elucidate the molecular mechanisms of fimbriae-mediated bacterial adhesion to host epithelial cells. This proposal focuses on the 987P fimbriae of enterotoxigenic *Escherichia coli* of piglets. The minor 987P fimbrial subunit FasG mediates bacterial binding to two types of receptors on piglet intestinal epithelial cells. Genetic and biochemical approaches will be used to identify and delineate the binding domains of FasG specific for each receptor. Mutants unable to bind either to one or to both receptors will be constructed by genetic engineering. Neonatal piglets will be infected with these mutants to determine the relative importance of each binding interaction in pathogenicity. Studies on the structure-function relationship of 987P will help to define its unique attributes as a carrier of separate adhesive domains, each specific for different complementary receptors on the relevant host compartment. In addition to serving as a paradigm for unraveling complex mechanisms of host-pathogen

recognition, studies on this system of veterinary relevance will support the development of improved preventive approaches against enteric infections in animals.

9702704 Mucus-inducible Genes and Proteins of *Vibrio anguillarum* as Vaccine Candidates
Nelson, D.R. **Grant 97-35204-4811**

University of Rhode Island **Strengthening Award**
Department of Biochemistry, Microbiology, and Molecular Genetics **$100,000**
Kingston, RI 02881-0800 **2 Years**

It has been shown that the fish gastrointestinal tract is a major portal of entry for the bacterium *Vibrio anguillarum*, the causative agent of vibriosis. Vibriosis is one of the most destructive bacterial diseases of fish. It causes significant losses to the aquaculture industry and can limit the production of finfish in seawater. Though normally considered an infection of marine fish, *V. anguillarum* can become established in fresh water with devastating results to freshwater fish. We have demonstrated that when this bacterium is grown in salmon intestinal mucus, new outer membrane proteins are produced. We hypothesize that these new mucus-inducible outer membrane proteins are necessary for growth in gastrointestinal mucus and may serve as vaccine candidates for the development of improved vaccines against vibriosis. We propose to: 1) clone and characterize one of the genes that encode a mucus-inducible protein (MIP) of *V. anguillarum*, 2) determine the function of the cloned MIP during growth in mucus; and 3) determine whether a mutant strain of *V. anguillarum* no longer able to make the MIP is avirulent, whether such a mutant may serve as a live vaccine strain, and whether the purified MIP may serve as a vaccine candidate. Information gained as a result of this investigation should enable the development of improved and more efficacious vaccines against vibriosis, as well as provide new methods for the development of vaccines against other potential fish pathogens. The development of improved vaccines will enhance the growth and economic success of the aquaculture industry.

9702622 Gordon Research Conference on Mycotoxins and Phycotoxins
Haschek-Hock, W.M. **Grant 97-35204-4300**

Gordon Research Conferences
University of Rhode Island **$5,000**
West Kingston, RI 02892-0984 **1 Year**

The Gordon Research Conference program fosters interdisciplinary scientific interchange and enhances productive interactions among scientists from around the world. Scientists from universities, government laboratories, and industry are given opportunities to interact on a formal and informal basis. The Gordon Research Conference on Mycotoxins and Phycotoxins is unique since it brings scientists with interest in either mycotoxins or phycotoxins, or both, and covers a wide range of topics ranging from basic chemistry to mechanisms of action and risk assessment. This conference meets every two years; the next meeting is June 15-20, 1997, in Henniker, New Hampshire. The goal of the conference is to advance knowledge and understanding in the area of mycotoxins and phycotoxins. Strong international participation is a feature of this meeting. At the last meeting, held in 1995, 36% of participants were from countries outside the USA, including Canada, Southern America, Europe, Asia, Australia and Africa.

Mycotoxins are naturally-occurring compounds produced by fungi which frequently infest grain crops worldwide. These compounds, for the most part, cannot be eliminated totally and thus are found in human foods and animal feed. This is especially true in developing countries where growing and storage conditions are often poor. Mycotoxins are known to affect animal and human health. These effects can be acute or chronic, and may include carcinogenesis. In addition to the direct economic costs of animal losses and production losses, mycotoxins are extremely good non-tariff trade barriers. Risk assessment and food safety issues in terms of human health are ongoing issues of public debate. This grant is for meeting support of scientists who will address current issues in the mycotoxin area including chemistry, toxin metabolism, emerging and re-emerging toxins/toxicoses, molecular epidemiology, neurobehavioural effects, and food safety.

9702751 Virological, Immunological, and Molecular Components of Reproductive PRRS
Rowland, R.R.R.; Benfield, D.A., Cafruny, W. **Grant 97-35204-5071**

South Dakota State University **Strengthening Award**
Department of Biology/Microbiology **$158,955**
Brookings, SD 57007 **3 Years**

Porcine reproductive and respiratory syndrome (PRRS) has emerged as the most economically important disease of swine in the 1990's. The infection of pregnant gilts and sows with PRRS virus (PRRSV) produces a spectrum of reproductive disease outcomes, ranging from fetal death and abortion to persistent infection of piglets. Even though abortion and stillbirths are the most noticeable effects, the impact of persistent infection is beginning to be realized. Ongoing work on persistent PRRSV following intrauterine infection suggests that PRRSV causes problems in the nursery and persists for several months and is eventually re-introduced into the next breeding cycle. The anatomy, reproductive physiology, and immunology of the pig provide an ideal

model for understanding how PRRSV causes reproductive disease. The overall goal of this project is to monitor changes in virus replication and immune cell cytokines at the maternal-fetal interface during the infection of pregnant pigs. This requires the use of a variety of techniques including virus isolation, polymerase chain reaction, in situ hybridization, immunohistochemistry and enzyme-linked immunoassay (ELISA). The first objective is to develop a disease model by comparing three strains of PRRSV that represent the whole range of PRRSV virulence. The second objective is to determine if persistently-infected female pigs pass PRRSV vertically to developing fetuses.

9703225 Reactivity of Bovine Vasculature to Ergovaline and Ergine of Toxic Tall Fescue
Oliver, J.W.; Schultze, A.E.; Linnabary, R.D.; Rohrbach, B.W. **Grant 97-35204-4814**

University of Tennessee
Department of Comparative Medicine
Knoxville, TN 37901-1071
$188,000
3 Years

Tall fescue toxicosis remains an ill-defined disease of herbivores that results in billion-dollar losses to U.S. agribusiness annually, with losses to the cattle industry alone exceeding eight hundred (800) million dollars. Results of preliminary studies reveal that several important changes occur in vascular tissues of cattle that consume the endophytic-fungus (*Neotyphodium coenophialum)* of tall fescue, including change in sensitivity of vascular receptors associated with vasoconstriction. Damage to the lining cells of blood vessels also occurs, and increased levels of chemical agents are present in the bloodstream that are associated with inflammation. The muscular layer of blood vessels is thickened, which together with the vasoconstriction, damaged lining cells and inflammatory mediator presence, contributes to altered blood flow that predisposes the animal to blood clotting events. The overall metabolic activity of the animal is also suppressed, as shown by reduced levels of selected serum analysts.

This research will focus on determining the comparative effects of the two important alkaloids in endophyte-positive tall fescue (Ergine, ergovaline) on vascular tissues of cattle. Both live animal and isolated cell culture approaches will be used to determine the prolonged effect of these two fescue toxins on the integrity and function of blood vessels. An improved understanding of the toxic mechanisms of alkaloids found in endophyte-positive tall fescue must occur, before safe and efficacious remedies can be developed for the disease. Knowledge of comparative effects of alkaloids on animals will also allow researchers to genetically manipulate fescue grass varieties to benefit producers.

9703055 Cytotoxic T Lymphocytes and IBV Pathogenesis
Collisson, E.W. **Grant 97-35204-5069**

Texas A&M University
Department of Veterinary Pathobiology
College Station, TX 77843
$160,000
2 Years

Not only is infectious bronchitis virus (IBV) a continuing economic problem in the poultry industry, the chicken is an ideal model for studying immunity to viral infection in animals. Outbreaks of IBV in chicken houses regularly occur in spite of the wide use of vaccines. In part, the difficulty in controlling this virus stems from the large numbers of distinct strains, each requiring their own vaccine. However, vaccine development has concentrated on inducing antibody based immunity which eliminates viral particles and prevents attachment of virus to host cells. In contrast, cellular immunity that targets the removal of virally infected cells has been, for the most part, neglected in current vaccine strategies. Unlike most antibody based immunity, cellular immunity to viruses typically also is able to target a broader spectrum of virus types. This work will characterize the cellular immune response that specifically removes or lyses cells supporting the propagation of IBV, that is cytotoxic T lymphocyte (CTL) immunity and identify viral components that will be evaluated and correlated with the presence of virus in the bird, specific cell types that are responsible for CTL immunity will be determined and the effects of antibodies and cells producing antibodies on CTL development after infection will be determined. Furthermore, the specific proteins in the virus, as well as fragments of these proteins in the virus, as well as fragments of these proteins, will be identified that induce CTL.

9703230 Clay-Based Strategies for the Prevention of Mycotoxicoses in Animals
Phillips, T.D.; Kubena, L.F. **Grant 97-35204-4813**

Texas A&M University
Department of Veterinary Anatomy and Public Health
College Station, TX 77843-0001
$165,000
3 Years

Aflatoxins, zearalenone, deoxynivalenol, and fumonisins comprise a diverse group of pervasive and naturally-occurring fungal poisons that are strongly implicated in disease and death in man and animals. Consequently, there is a critical need for practical methods of detoxification. An understanding of the fundamental chemical basis involved in mycotoxin adsorption to phyllosilicate clay surfaces will expedite the long-term goals of this research, i.e., the identification of safe and effective clays for the prevention of mycotoxicoses in animals and mycotoxin residues in food of animal origin. Clay and zeolitic minerals possess distinct structure

and are not created equal in their propensity to adsorb mycotoxins. Important structural differences provide unique functional and chemical properties. Because of a lack of purity and homogeneity in clay deposits, it is critical to be able to accurately characterize the binding performance of different clays. The inclusion of nonselective clays in feeds is risky! The main goal of this project is to develop model *in vitro* methods to accurately predict *in vivo* performance and the safety of diverse clays and "*mycotoxin binders*". Mycotoxin contamination of animal feeds routinely threatens the health of animals and humans. Optimal strategies to neutralize these poisons based on the inclusion of mycotoxin-selective clays in the diet, represent an important advance in technology with consequential and wide-ranging implications for the improvement and advancement of animal health.

9702658 Design and Characterization of MSCRAMM-Based Vaccines
Patti, J.M. **Grant 97-35204-5046**

Texas A&M University Institute of Biosciences and Technology
Center for Extracellular Matrix Biology **$210,000**
Houston, TX 77030 **2 Years**

Bovine mastitis is the most economically important disease to the dairy industry. *Staphylococcus aureus* is known to be the predominant mastitis causing pathogen worldwide despite improved management practices, germicidical teat dips, and antibiotic therapy. Attachment of the bacterium to bovine epithelial cells and the subsequent colonization of mammary gland tissues are the critical steps in the development of mastitis. Colonization of the mammary gland involves the attachment of bacteria to host tissues and microbial cell surface components recognizing adhesive matrix molecules (MSCRAMMs) are responsible for mediating this adherence. Microbial adhesion is often required for bacterial survival, in addition it helps the bacteria to evade host defense mechanisms and antibiotic challenges. The increasing number of antibiotic resistant staphylococci and higher demands for residue free milk, coupled with a lack of new therapeutics in clinical trials, indicate the need for alternative strategies to prevent *S. aureus* related infections. The goal of this proposal is the development, characterization, and testing of MSCRAMM-based vaccines. We expect this information will form the basis for the design of novel vaccines that can be used to prevent and treat *S. aureus* mediated bovine mastitis. Moreover, since adherence to host tissue components is a prerequisite for the initiation of infections by virtually all pathogenic microorganisms, these findings will provide a strong platform for the development of vaccines against other animal infectious diseases.

9702628 Neuroendocrine Mechanisms of Central CRH and Stress-Induced Behavior and Immune Changes
McGlone, J. **Grant 97-35204-4810**

Texas Tech University
Department of Animal Science & Food Technology **$162,000**
Lubbock, TX 79409-2141 **3 Years**

Evaluation of farm animal well-being remains a challenge to the scientific community. Most workers in the area agree that some combination of physiology, behavior and cognitive experiences of the animal are the best indicators of animal well-being. The basic neural mechanisms that are the underpinnings for behavioral, physiological and cognitive responses indicative of animal well-being remain elusive. The model we propose involves application of the stress hormone corticotropin releasing hormone (CRH) directly into the brain of freely-moving pigs. This treatment causes an immediate and dramatic effect on pig behavior and endocrine and immune systems, resembling the physiology and behavior of a stressed pig. The behavioral and immune effects of central CRH could be mediated by peripheral or brain endocrine signals yet to be determined. This proposal addresses two objectives towards improvement of our understanding of neuroendocrine mechanisms: (1) To describe the CRH dose-response relationship between stereotyped behaviors add immune measures and (2) To determine the effects of antagonizing certain hormones while brain CRH is given that is known to influence pig behavior and immune measures. With these results in hand we will (1) have a better understanding of neuroendocrine mechanisms of CRH and stress-induced changes in behavior and immune events and (2) be in a position to improve pig well-being through interventions designed to reduce anxiety, and improve pig health during unavoidable times of stress.

9703081 Preventing Mycotoxin Disease in Poultry by Dietary Induction of Glutathione S-transferases
Coulombe, R.A.; Buckner, R.E.; Frame, D. **Grant 97-35201-4959**

Utah State University **Strengthening Award**
Department of Animal, Dairy, and Veterinary Sciences **$163,802**
Logan, UT 84322-4620 **3 Years**

Mold-produced toxins are unavoidable contaminants of poultry feeds. Aflatoxin B1 (AFB1) is the most important mycotoxin with respect to occurrence and toxic potency, and poultry are the most susceptible food animals to it's effects. The annual economic impact of aflatoxin -related diseases to the poultry industry exceeds $100 million. Small amounts of AFB1 cause reductions in growth rate, feed efficiency, hatchability and increased susceptibility to bacterial and viral diseases. We have recently discovered that the extreme sensitivity of turkeys to AFB1 is primarily due to a deficiency of the toxin-fighting enzyme,

glutathione S-transferase (GST). However, our studies have shown that, as with many mammalian species (including humans), the expression and activity of this important enzyme can be increased in turkeys by a simple addition of the FDA-approved antioxidant butylated hydroxytoluene (BHT) to the diet. We will confirm whether this and related dietary antioxidants shown to increase AFB1-detoxifying GSTs in mammals will do so in turkeys, and if these antioxidants will protect turkeys against AFB1-related toxicity. Because of the important role GST plays in protecting animals against both natural and synthetic toxins, we wish to characterize turkey GSTs. Dietary interventions with antioxidants and other chemoprotective agents is now recognized to be one of the most promising cancer-prevention strategies in people. Since AFB1 and other mycotoxins are unavoidable in feeds, discovering ways of protecting turkeys through simple dietary intervention with safe chemoprotective agents represents a practical management strategy that should be explored. Thus, our research will help to significantly reduce the losses associated with mycotoxins in poultry feeds, thereby helping the poultry industry to be more productive and to produce safer food for consumers.

9703227 Prostacylcin Production by Uterine Arteries in Response to Lipopolysaccharide
Vagnoni, K.E. **Grant 97-35204-4912**

Utah State University **Strengthening Award**
Department of Animal, Dairy, and Veterinary Science **$140,000**
Logan, UT 84322-4700 **2 Years**

Anecdotal and clinical evidence suggest that female domestic animals vary in their response to noxious agents depending on the stage of their reproductive cycle. Exposure to one such noxious agent, lipopolysaccharide (the lipo-carbohydrate molecules which make up the cell wall of certain bacteria), commonly results in overreaction by the immune system (a condition called endotoxemia) which is often followed by abortion or death of the animal. Evidence exist for a regulatory role of the female sex steroid, estrogen, on the immune response to lipopolysaccharide. The research proposed in this study will determine the role of estrogen in regulating the response of sheep arteries to lipopolysaccharide. These studies will determine if estrogen's influence on immune response is limited to reproductive associated tissues (i.e., uterine arteries) or whether estrogen can influence tissue throughout the body (for example, arteries of the kidney). In addition, these studies will determine the cell population within arteries that respond to lipopolysaccharide. Data collected from these studies will provide useful information for determining the optimal timing for immunization of females and for better management of females once exposed to a noxious agent such as lipopoly saccharide. In addition, identification of the cell population which responds to lipopolysaccharide will provide useful information for development of vaccination and treatments which specifically target responding cells, thus increasing the effectiveness of these treatments. Taken together, data from these studies, when applied to management schemes, will allow further enhancement of animal health and well-being.

9702285 *Brucella abortus* Vaccine Strain RB51: Improvement and Development of a Multivalent vaccine
Schurig, G.G.; Boyle, S.M. **Grant 97-35204-4483**

Virginia Polytechnic Institute and State University VA-MD Regional College of Veterinary Medicine
Department of Biomedical Sciences and Pathobiology **$120,000**
Blacksburg, VA 24061-0442 **2 Years**

Brucella abortus vaccine strain RB51 has now replaced strain 19 as the vaccine against Brucellosis in the USA. The major advantage of strain RB51 over strain 19 is that it will not confuse the diagnosis of the disease and therefore, accelerate eradication of the disease at a lower cost. Like strain 19, strain RB51 is highly attenuated and stable and is used as a live vaccine. Its protective abilities are similar to strain 19; neither strains 19 nor RB51 can protect 100% of the vaccinated animals from Brucellosis. For complete eradication of the disease from cattle and wildlife the use of a more effective vaccine would be advantageous. This project intends to make strain RB51 close to 100% effective by increasing its output of protective substances (antigens). Pilot studies in mice indicate that when vaccine strain RB51 overproduces a protective antigen, it's protective efficiency is close to 100% and it's degree of attenuation is not changed. Pilot studies also indicate that strain RB51 can produce antigens from unrelated bacteria. This project will also attempt to have strain RB51 produce protective antigens from other bacteria in addition to overproducing its own. We will attempt to produce a *Brucella* vaccine close to 100% efficient against Brucellosis and able to protect against a major disease like tuberculosis. Therefore, one vaccine would protect effectively against two diseases saving labor and cost to the producer and ultimately, bring a better and cheaper product to the consumer's table.

9700497 Development and Comparative Immunology Congress
Kaatari, S.L. **Grant 97-35204-4120**

College of William and Mary Virginia Institute of Marine Science
Department of Environmental Sciences **$9,000**
Gloucester Point, VA 23062-1204 **6 Months**

The International Society for Development and Comparative Immunology (ISDCI) was founded in 1978 to bring together investigators studying immunological processes in all animal species, with particular concentration upon fish, other non-

mammalian vertebrates, and invertebrates. To achieve this objective, ISDCI convenes an international congress every three years and assists in the organization of additional international conferences, symposia, workshops, and training courses each year. The primary objective of the Seventh Congress of the ISDCI is to serve as a vehicle for the presentation of the most current research of the membership of the Society and those scientists who possess similar interests. Of particular pertinence to the mission of the NRICGP of the USDA is the focus of a number of sessions on the topics of disease resistance in aquaculturally and agriculturally important animals, the breeding and understanding of the genetics of disease resistance in these animals, the development of immunological techniques for the monitoring of diseases, and the analysis of disease susceptibility and resistance in insect vectors and agricultural pests.

9702468 Plasmin/Plasminogen System and Mammary Infection
Bramley A.J.; Zavizion, B. **Grant 97-35204-4485**

University of Vermont
Department of Animal and Food Sciences
Burlington, VT 05405
$100,000
2 Years

Despite intensive research and the introduction of control programs, mastitis has remained the most economically damaging disease for dairy cattle worldwide. Additional progress using standard methods does not seem likely. Therefore, the primary goal of the proposed research is to provide an improved understanding of the molecular mechanisms involved in the pathogenesis of bovine mastitis caused by *Staphylococcus aureus*. The current focus is on the principle pathways by which this pathogenic bacteria may utilize the host proteolytic system to aid colonization, survival, and multiplication in the bovine mammary gland. Particularly it includes the fundamental investigation of the ability of *S. aureus* to modulate the plasminogen activator system in bovine mammary epithelial and myoepithelial cells. From our preliminary data it is apparent that *S. aureus* increases plasminogen activator activity in cell culture, and this aids migration of staphylococci across mammary epithelial cell monolayers. Thus, it is likely that the recruitment of the host plasmin/plasminogen activator system could be a significant virulence and invasion factor for bovine staphylococci. This study is designed to investigate and identify specific bacterial product(s) responsible for activation of the plasminogen activator system. The results of this investigation will be useful tools in the future development of immunoprophylactic, and immunotherapeutic approaches for this type of infection.

9702344 Cytokine Modulation of Reciprocal T Cell-APC Signaling
Brown, W.C.; Palmer, G.H.; Rice-Ficht, A.C.; Estes, D.M. **Grant 97-35204-4513**

Washington State University
Department of Veterinary Microbiology and Pathology
Pullman, WA 99164-7040
$260,000
3 Years

Understanding how immune responses are regulated in ruminants is critical for devising strategies to direct an immune response towards a desired effector function required for either preventing infection or eliminating disease. For many infectious diseases, it would be advantageous to induce both a type 1 cytokine response (i.e. macrophage activating IFN-g) and strong humoral immunity. For use as vaccine adjuvants, the capacity of cytokines to either suppress T cell responses or to prevent B cells and T cells from undergoing activation-induced cell death during cognate T cell-antigen presenting cell (APC) interactions must be determined. Different pathogens and their antigens can induce IL-10 in macrophages and antigen-specific T cells, causing antigen-specific unresponsiveness. By inhibiting the induction of co-stimulatory B7 molecules on APC, IL-10 down regulates antigen-specific immune responses, and in its absence, inflammatory T cell responses remain unchecked. This proposal focuses on understanding the role and regulation of death and co-stimulatory molecules in effector cell responses involving both T cell-B cell interactions required for the development of T cell dependent antibody responses, and T cell-macrophage responses required for the development of inflammatory immune responses. First, we will determine whether helper T cells that express the death molecule, Fas ligand, and IFN-g in the absence of IL-4 can act as helper cells for cognate T cell-dependent antibody responses to induce Fas-expressing B cells to produce IgG2. Second, we will determine the molecular mechanism by which IL-10 down regulates T cell function through it effect on B7 co-stimulatory molecules on macrophage and B lymphocyte APCs.

9700001 Cytokines and the Type I Type II Paradigm
Davis, W.C.; Brown, W.C. **Grant 97-35204-3952**

Washington State University
Department of Veterinary Microbiology and Pathology
Pullman, WA 99164-7040
$8,500
6 Months

Cytokines and their receptors have become a major focus of research in humans as well as food and companion animals because of their central role in the regulation of cellular and humoral immune responses. The insights gained from studies on the function of these molecules suggest they may, alone or in combination, prove useful as agents to selectively alter the development of an immune response to pathogens and parasites and derived submit vaccines. In addition, cytokines and their receptors may

prove useful as therapeutic agents to selectively alter or redirect immune responses harmful to the host. The advances made in analysis of the expression of cytokines in different species have now made it clear that both αβ and γδ T cells secrete different combinations of cytokines following stimulation, indicating that both populations of cells are involved in regulation and expression of immunity. An international symposium has been organized to provide an opportunity for investigators to review the current status of knowledge on cytokines and their receptors in humans, in food and companion animals, and in laboratory animals, and to exchange theories, methodology, and new data on the role of cytokines secreted by αβ and γδ T cells in the regulation of immune responses. The interaction of leading scientists at the meeting will lead to new insights into how cytokines and their receptors interact and function during the development and expression of immune responses and the development of methods to use cytokines to improve animal and human health.

9702317 Mechanisms Controlling the Expression of Cannibalism in the Domestic Fowl
Newberry, R.C.; Ulibarri, C.M. **Grant 97-35204-4812**

Washington State University, Pullman
Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology **$290,000**
Pullman, WA 99164-6520 **3 Years**

Cannibalism is a poorly understood behavior that adversely affects the well-being of laying hens and causes economic losses to American poultry producers. The usual method of controlling cannibalism, beak trimming, is a procedure that also raises concerns about hen well-being. The goal of this study is to identify the underlying causation of cannibalism in laying hens, leading to methods of controlling this behavior without beak trimming. This outcome is important to the future sustainability of the American egg industry, especially since animal well-being issues may become the source of future non-tariff trade barriers. The research is designed to determine whether cannibalism is a form of competitive behavior related to aggression or a form of foraging behavior that is redirected to other birds due to inadequate foraging opportunities. The research will also establish whether individual birds have behavioral or physical traits that can be used to predict whether they will become cannibalistic or susceptible to cannibalistic attack. Furthermore, the study will assess whether there is any association between cannibalistic behavior and low brain serotonin activity. These goals will be pursued in three experiments investigating effects of: (1) group size and stocking density, (2) introducing unfamiliar birds into the flock and supplementing the diet with tryptophan to increase brain serotonin levels, and (3) feeding less concentrated and more varied diets. By improving knowledge about the causation of cannibalism, this research will facilitate the development of humane control methods and contribute to improved poultry well-being and sustainability of US poultry production.

9702488 Importance of Emergent Genetic Variants in Rickettsial Disease Outbreaks
Palmer, G.H.; McElwain, T.F. **Grant 97-35204-4597**

Washington State University
Department of Veterinary Microbiology and Pathology **$240,000**
Pullman, WA 99164-7040 **3 Years**

Anaplasma marginale is the most prevalent rickettsial pathogen of cattle and causes severe morbidity and mortality following tick-borne transmission from asymptomatic persistently infected cattle. Transmission, however, does not occur continuously or predictably despite commingling of infected and susceptible cattle in the presence of tick vectors. This unpredictability results in either unnecessary expenses due to frequent acaricide application or continual antibiotic feeding, or, more commonly in beef herds, failure to implement control measures resulting in periodic disease outbreaks with severe economic loss. We propose that identifying the determinants responsible for transmission is necessary to improve strategic application of current control methods and to facilitate development of novel methods which target the critical points in transmission. The goal of this study is to determine how tick-borne rickettsial outbreaks occur with the aim of improving disease control. Using retrospective analysis of outbreaks, we identified a unique genotype associated with each outbreak. We hypothesize that emergence of a unique genotype is associated with a high level and rate of tick infection and is responsible for increased transmission leading to disease outbreaks. The hypothesis will be tested prospectively in infected herds with endemic regions. Using genotypic analysis, we will determine whether disease outbreaks within a herd are associated with emergence of a genetic variant distinct from the strains currently present in the herd. How an emergent genetic variant results in increased transmission will be determined by testing the effect of the variant on three key transmission variables: 1) enhancement of the rickettsemia level in persistently infected cattle which serve as infection reservoirs; 2) enhancement of tick infection; and 3) enhanced infectivity for susceptible cattle.

9702262 Mechanism of control of *Anaplasma marginale* Rickettsemia
Wyatt, C.R.; McGuire, T.C. **Grant 97-35204-4915**

Washington State University
Department of Veterinary Microbiology and Pathology
Pullman, WA 99164-7040
$180,000
2 Years

Anaplasmosis is caused by the rickettsial hemoparasite, *Anaplasma marginale*, and infects cattle worldwide. Acute anaplasmosis is characterized by anemia and weakness, and by abortion in pregnant cows, Cattle that recover from acute disease remain persistently infected, and serve as potential reservoirs of infection for other cattle. Efforts to produce efficacious vaccines have not been successful, in part because of a lack of sufficient information about host responses to the organism. The purpose of this study is to evaluate a potential mechanism for control of acute anaplasmosis in cattle. Peripheral blood lymphocytes from cattle recovering from acute disease can be stimulated by *A. marginale* in culture to secrete a molecule that kills the parasite in the red blood cell. This observation suggests that a potential mechanism for control of parasitemia might involve an immune response aimed at killing the parasite in red cells. The identity of this molecule is unknown, as are the optimum conditions for its secretion. Further, the time of appearance and duration in blood of the cells that produce this factor are not well defined. The goals of this project are to identity the time point during acute infection when lymphocytes from affected cattle can be induced to produce this rickettsicidal factor, and to isolate and characterize the factor. Completion of this research will provide new and important information that will contribute to vaccine development aimed at enhancing protective immunity to hemoparasites in domestic animals.

9702403 DNA Vaccines for Salmonid Fishes
Kurath, G.; Anderson, E.D. **Grant 97-35204-4735**

USDI United States Geological Survey
Northwest Biological Science Center
Seattle, WA 98115-5016
$184,000
3 Years

Due to continued demand for seafood products and increasing restrictions on water use, enhanced productivity of aquaculture will be required in the future. However, when fish are confined and intensively reared, infectious diseases can be devastating. Thus, vaccines will be a vital component in sustaining development of the aquaculture industry. Infectious Hematopoietic Necrosis Virus (IHNV) causes a sudden, lethal disease in many species of salmon and trout, and is the most costly viral fish pathogen in North America. Although IHNV outbreaks have occurred in salmon hatcheries throughout the Pacific Northwest for decades, there is no commercially available vaccine against IHNV due to cost or low efficiency. A new technology, DNA vaccines, has been developed recently as an alternative to traditional vaccines. In preliminary work, a prototype DNA vaccine against IHNV was shown to trigger an immune response in fish and protect them from IHNV disease. The current proposal will expand on this observation with vaccine optimization studies on minimal dose, fish size, different vaccine constructs, effectiveness against numerous strains of IHNV, and duration of the protective response. Detailed studies on the biology and immunology of the response to DNA vaccination in fish will provide information on how these vaccines function. These studies lay the groundwork for development of the first fish DNA vaccine, and comprise a model system for understanding DNA vaccines in fish. Once understood, transfer of this technology for development of vaccines against other significant viral, bacterial, and protozoan pathogens of fish will be possible.

9703063 Oral Immunization with Yeast Expressing Anchored BHV1 gD and Bovine Cytokines
Letchworth, G.J. **Grant 97-35204-4737**

University of Wisconsin, Madison
Animal Health and Biomedical Sciences
Madison, WI 53706-1581
$200,000
2 Years

Bovine herpesvirus-1 (BHV1) causes costly disease in cattle worldwide. It has been difficult to control or eradicate because it always persists in convalescent animals. Existing vaccines do not prevent infection or viral persistence. The PI has shown that an intranasal aerosol of purified BHV1
gD stimulates the bovine mucosal immune system to produce nasal mucus antibodies that form a mucosal barrier against infection. However, this method of immunization is impractical and fails to stimulate solid immunity in all animals. Recently, the PI's laboratory has engineered yeast cells to secrete the gD extracellular domain. The yeast protein engenders neutralizing antibodies in mice. Also, yeast cells have been proven by others to express biologically active ruminant cytokines. The goal of this project is to test the hypothesis that a yeast cell wall anchorage molecule called alpha-agglutinin can tether the external part of BHV1 gD and the bovine cytokines IL-5, and IL-6 to the surface of yeast cells to create a surface capable of engendering a protective mucosal immune response. The BHV1 gD would stimulate antibodies against the virus and the bovine cytokines on the yeast cell surface should act as an adjuvant directing the host immune system toward mucosal immunity. We expect that animals vaccinated orally with recombinant yeast will develop a high and durable antibody response in all mucosal surfaces. Success in this research

would lead to oral vaccines for other animal and human viruses in which envelope glycoproteins are the targets of protective immunity.

9702627 V(D)J Recombination During Bovine Fetal Development
Halligan, B.D. **Grant 95-37204-2188**

Medical College of Wisconsin
Department of Microbiology
Milwaukee, WI 53226-0509

$155,00
2 Years

The most important event in mammalian immune development is the process of antibody and T-cell receptor gene segment rearrangement. This process, known as V(D)J recombination, is responsible for the generation of the near infinite number of different genes encoding the antigen receptor proteins and is required for the successful completion of the lymphoid developmental program. Almost all of our knowledge of the regulation of this critical process in lymphoid development comes from the study of virus transformed mouse fetal liver cell lines, a model system that may be less than perfect, and not representative of lymphoid development in agriculturally relevant species. We propose to continue our examination of lymphoid development in the fetal bovine system using the expression of proteins thought to play a role in V(D)J recombination as a measure of V(D)J recombinational activity.

Our specific aims will be to clone additional genetic probes for the proteins involved in V(D)J recombination from cattle and use these probes, as well as biochemical assays, to determine at what stages of fetal life V(D)J recombination occurs in cattle.

From these experiments, we will not only significantly enhance the understanding of the development of the immune system in an agriculturally significant organism, the cow, and obtain molecular clones and cDNA sequences for bovine proteins that are involved in V(D)J recombination that can be used by other investigators, we will also determine if the current understanding of lymphoid development during the fetal period is correct.

9702405 Mechanisms of *Streptococcus suis* Meningitis
Drevets, D.A. **Grant 97-35204-4486**

West Virginia University R.C. Byrd Health Sciences Center
Department of Medicine
Morgantown, WV 26506-9163

Strengthening Award
$110,000
2 Years

Streptococcus suis is pathogenic bacterium found in swine herds throughout the world for which an effective vaccine is not yet available. It is a major cause of severe infections in young swine including sepsis, serositis, and meningitis. One mechanism used by microbes to invade protected spaces such as the central nervous system is for circulating bacteria to bind vascular endothelial cells and then enter them. Bacterial infection of endothelial cells in turn stimulates a host response including production of pro-inflammatory cytokines and upregulated expression of endothelial cell adhesion molecules. Current models of bacterial meningitis show that these responses are critical for enabling leukocyte recruitment and entry into the central nervous system. Despite growing knowledge of inflammatory mediators and leukocyte endothelial cell adhesion mechanisms, little information exists about basic mechanisms of inflammation in *S. suis* meningitis. In this project we will characterize production of pro-inflammatory cytokines systemically and in the brain during experimental *S. suis* infection. We will test whether the expression of specific brain microvascular endothelial cells in vitro, and determine whether endothelial cell infection alters their adhesion molecule expression or their adhesiveness for neutrophil and monocytes. The in depth study of the host response to this bacterium may ultimately lead to opportunities to intervene in the disease process though drugs other than antibiotics, or by identifying new targets for vaccine development.